DRIVERS OF GRASSLAND BIODIVERSITY IN THE SWISS ALPS

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General Introduction

This thesis is a comprehensive study of biodiversity at all levels of biological integration in mountain grasslands in the Swiss Alps. It evaluates the relative importance of potential drivers of biodiversity in the alpine landscape, such as land use and altitude, and in Switzerland specifically cultural traditions. Identifying drivers of biodiversity at all levels (community, biological interactions, species, and genes) creates the basis for political measures to protect and promote biodiversity. Moreover, drivers of biodiversity might act opposingly upon different levels of biodiversity and thus evoke potential conflicts not only between different levels of biodiversity, but also between biodiversity and agriculture. In this general introduction, I provide background knowledge and conclude with an outline of this thesis.

Background

Biodiversity

The United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro in 1992 recognized the importance and the value of biodiversity in the Convention on Biological Diversity (UNCED 1992). The contracting 170 countries are committed to report, monitor, and conserve their biological diversity and to sustainably use their biological resources. Biodiversity consists of several levels of biological integration of equal importance: the diversity of habitats and ecosystems across geographical regions, community diversity including biological interactions such as pollination, herbivory and plant-fungal associations, species diversity, and genetic diversity within species (Primack 1993). Moreover, these levels of biodiversity need to be considered at different spatial scales, within (alpha-diversity) and between (beta-diversity) study sites (Primack 1993). While comprehensive conservation needs to be based on thorough knowledge of biodiversity at all these levels and spatial scales, comprehensive studies providing such knowledge are very scarce.

Several aspects underline the significance of biodiversity and justify efforts for its protection: Biodiversity enhances the functioning and the stability of ecosystems (Hector et al. 1999) and improves the sustainability of resource use (Klaus et al. 2001). Moreover, higher biodiversity strengthens stress tolerance of organisms and protects ecosystems against disturbances (Hooper et al. 2005). Furthermore, the protection of biodiversity is important for

ethical reasons such as every individual's right of existence (Aus der Au 2003). Finally, as meadows of high plant species diversity have higher perception values for people as those of low diversity (Junge 2004), aesthetic reasons complete the significance of biodiversity.

Ecology and economy are complexly linked in the conservation of biodiversity as biodiversity usually benefits the society, whereas the costs of its protection and preservation fall upon the individual (Edwards and Abivardi 1998). Moreover, different perception by different stakeholders complicates the protection of biodiversity (Baur 2004). Tourists and walkers enjoy the floridity of mountain meadows used at low-intensity, whereas farmers chiefly count on the yield. The lower the yield and the more laborious to manage an agricultural parcel of land, the more likely it will be left abandoned. For some decades, our landscapes have developed towards more and more intensively used fields close to villages and farms, either by using more fertilizer or by higher stocking rates (Bätzing 2003). Areas that are far from housing areas or farms, or are difficult to reach for topographical reasons are more and more left abandoned. In turn, they develop into dense grass stands or they are taken over by forest leading to a dramatic decline in grassland area. In either case, biodiversity decreases.

Alpine biodiversity

The variety of climatic conditions, topography, and ecosystems characterize potential drivers of biodiversity in the Alps. Not only various combinations of physiographic factors shape the biodiversity of mountain areas but also human management systems (Nagy 2003). A recent inventory of alpine biodiversity in Europe focused on taxonomic richness of plant and animal communities including their pattern and diversity in space and time, and the underlying ecosystem processes above timberline of different European mountain systems (Nagy 2003). In all mountain systems, plant species richness was found to decrease with elevation (Theurillat et al. 2003). A variety of ways to cope with alpine conditions was shown for groups of insects and other arthropods. For butterflies, habitat type and grazing impact was found to be more important than altitude (Tontini 2003). However, all studies focused on taxonomic levels of species diversity and did not consider other levels of biodiversity pointing out the necessity of a comprehensive study comprising all levels of biodiversity.

Land use

For thousands of years, the landscape of the Alps has developed from a solely natural landscape of dominating dense forests to the actual cultural landscape characterized by a

diversified mosaic of pastures, meadows, and forests. The Swiss Alps have been influenced by human land use for about 5000 years (Bätzing 2003) resulting in man-made grasslands which replace cleared forests below timberline. These grasslands are characteristic for the cultural landscape of the Alps and contribute to creating one of the most plant species rich areas in Europe (Väre et al. 2003). Plant species diversity in man-made grasslands is up to three times higher than in the forests they are replacing (Zoller and Bischof 1980) and may depend on the type and intensity of land use regimes. The types of land use differ in stocking rates, cutting frequencies, and levels of fertilization. Some grassland is fertilized, often with liquid manure or dung, other remains unfertilized. Pastures are grazed either only for a few weeks or during the whole summer, mainly by cattle, but sheep and goats also occur frequently, especially at higher altitudes. Grazing intensity often varies among years depending on the climatic conditions. Unfertilized meadows are cut once a year, fertilized meadows up to four times during one vegetation period.

Current socio-economic changes are bringing about severe land use changes, mainly intensified use by applying fertilizers or the opposite by abandonment, which may both negatively affect biodiversity. However, up to date rarely any attention has been paid to the relative importance of drivers of biodiversity compared to topographic determinants. Moreover, despite the long history of grassland use in the Alps, the effect of socio-economically motivated changes in land use on the biodiversity of grassland in the Alps has not been studied. We hypothesize that grassland biodiversity is declining because of land use changes.

Cultural traditions

In the Swiss Alps, there are three main cultural traditions named after the original settling tribes. The Romanic culture developed with the increasing human population in Europe after 1800 BC, when settlements and land use were extended into Alpine valleys. It is characterized by a self-sufficient alpine economy practicing mixed farming with permanent compact villages at the valley bottoms, transient summer settlements at higher altitudes, and pastures above timberline (Im Hof 1986). Due to frequently practiced hereditary partitioning of parcels a small-grained and frequently terraced parcel structure evolved which is still visible in many Romanic villages (Bätzing 2003).

The Germanic culture developed after 600 AD, when Alemannic people from northern areas immigrated into the Alps and settled in the wetter northern and northeastern parts. Germanic villages typically consisted of scattered single farm houses surrounded by fields

and meadows. The farmers practiced mainly dairy farming, whereas permanent fields for grain cultivation were only rarely found. As hereditary partitioning of parcels was uncommon, the farm was inherited as a whole by a son of the farmer, and thus farms and parcels were usually larger than in Romanic villages (Bätzing 1991).

After 1200 AD, the Walser – Alemannic people from what today is the Swiss canton Valais – migrated eastwards through the Alpine belt as far as today's Austrian federal state Vorarlberg. They settled at relatively high altitudes, as lower parts of the valleys were already occupied. Therefore, Walser people did not cultivate grain and apart from farming they lived on trade and maintenance of alpine passes (Bätzing 1991).

Despite important changes during the last century, socio-economic differences are still remarkably pronounced among cultural traditions (Pfister 2004). We hypothesize that these socio-economic differences have different effects on land use at different altitudes. Therefore, we expect that differences among cultural traditions are still apparent nowadays in different current land use regimes and their effects on grassland biodiversity.

National Research Program 48 "Landscape and Habitats of the Alps" (NRP 48)

The National Research Program "Landscapes and Habitats of the Alps" of the Swiss National Science Foundation comprises 35 projects that analyze and evaluate economic, cultural, and ecological processes concerning landscape and habitat dynamics in the Alpine region (NFP48 2005). These inter- and transdisciplinary research projects aim at acquiring the knowledge on goals and actions needed for sustainable landscape development that is socially desired as well as economically acceptable and feasible for politics. The results will contribute to implement norms for the sustainable use and shaping of landscapes and habitats of the Alps. The overall aim of the program is to increase and promote the public awareness of the collective goods of landscape and habitat with their extensive social significance. Land use and biodiversity are two of the major issues of the NRP and are dealt with in several projects. Within the NRP our project is particular in so far as we consider all levels of biodiversity from the landscape to the gene to address the complexity of biodiversity. Moreover, we are the only project considering the effects of different cultural traditions on the cultural landscape of the Swiss Alps.

Outline of this thesis

To comprehensively study natural and anthropogenic effects on all levels of biodiversity we set up a hierarchical parcel-based design. In 12 villages in the Swiss Alps, four of each of the three cultural traditions Romanic, Germanic, and Walser, we selected grassland parcels at three altitudinal levels, in the valley (about 1000 m asl), at intermediate altitudes (in some regions called "Maiensäss", about 1500 m asl), and at the alp level (about 2000 m asl). We searched for grassland parcels that were characterized by the combination of different altitudes, management regimes (traditionally mown or grazed, and currently mown, grazed or abandoned) and fertilization status (fertilized or unfertilized). Finally, this procedure led us to select 216 grassland parcels, representing characteristic management regimes according to the altitudinal level and the village. All or a subset of these grassland parcels were the study objects of the six chapters of this thesis.

Chapter 1 addresses the level of plant species diversity. We ask whether cultural traditions, altitude, type of land use affect the likelihood of occurrence of a certain type of grassland parcel, plant species diversity, and productivity. Moreover, we ask whether the diversity of land use types affects plant species diversity in a village. To analyze the relationship between plant species diversity of grasslands, cultural traditions, land use and altitude, we took two vegetation records per parcel in randomly selected 5 m x 5 m plots. We estimated the ground cover percentage of all vascular plant species and counted the number of species per record. For each parcel, we calculated the mean ground cover for each species and obtained Shannon's index of diversity and Evenness. Moreover, we estimated productivity in the plots by harvesting standing crop 4 cm above ground in a randomly selected sub-area of 0.5 m x 0.5 m.

Chapter 2 covers the level of biological interactions between plants, herbivores, and fungal pathogens. We used the extent of leaf damage by herbivory and fungal pathogen infection as measure of level and diversity of plant-herbivore and plant-fungal pathogen interactions of grasslands in the Swiss Alps. We recorded the percent leaf area damaged by ten types of herbivory and five types of fungal pathogen infection on 12'054 plant leaves of legumes, other forbs, and graminoids collected in 215 grassland parcels of different land use and altitude in the Swiss Alps. We asked how the amount of leaf damage by and the diversity of, different types of herbivory and fungal pathogen infection are affected by land use and altitude. Furthermore, we analyzed how and whether leaf damage and diversity types are related to each other, to plant species richness, and to standing crop per grassland parcel.

The following four chapters are dedicated to the genetic diversity of *Poa alpina*. **Chapter 3** firstly relates the occurrence of the species to land use types and altitude. Secondly, we report the results of a quantitative field experiment with two plants of each of 615 genotypes, originating from 58 grassland parcels and 21 natural sites performed in a common garden. We measured several vegetative and reproductive traits and present the analyses to answer the question whether plant performance in the common garden suggests local adaptation of *P. alpina* to land sue and altitude.

In **Chapter 4** we characterize five microsatellite loci in *P. alpina*. Five of the 15 microsatellite inserts turned out polymorphic among eight plant species and allowed us to study molecular genetic variation. Chapter 4 presents a first application of these microsatellite markers and is the prerequisite of the subsequent Chapter 5.

In **Chapter 5** we used these five microsatellite markers to study the genetic diversity of 415 plants of *P. alpina* originating from 54 agriculturally used grassland parcels and 20 natural sites. We asked whether and how altitude, cultural traditions, and agricultural land use have affected genetic differentiation between populations of *Poa alpina* L.. Moreover, we ask how these potential determinants of biodiversity have affected genetic diversity within parcels, estimated as numbers of alleles per plant and per parcel.

Chapter 6 extends the study of determinants genetic diversity of *Poa alpina* to quantitative genetic diversity of *P. alpina*. We asked whether and how quantitative genetic diversity of *P. alpina* is affected by abiotic factors and by land use, altitude and cultural traditions. Moreover, integrating the results of all studies of this thesis we asked how different levels of biodiversity are related to each other. For this purpose, landscape diversity was measured as the number of different land use types per village, plant species diversity was obtained from the vegetation records of Chapter 1, the diversity of plant-herbivore and plant-pathogen interactions was obtained from Chapter 2, and microsatellite diversity from Chapter 5. As measures for quantitative genetic diversity, we used broad-sense heritability of several vegetative and reproductive characters obtained from the common garden experiment.

In the concluding summary I provide a short survey of the most important findings of this thesis and perspectives for research and conservation.

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1

Old cultural traditions, in addition to land use and topography, are shaping plant diversity of grasslands in the Alps

(submitted)

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Abstract

Socio-economically motivated land use changes are a major threat for species diversity of grasslands throughout the world. Here, we comprehensively explore how plant species diversity of grasslands in the species-rich cultural landscape of the Swiss Alps depends on recent land use changes, and, neglected in previous studies, old cultural traditions. We studied diversity in 216 grassland parcels at three altitudinal levels in 12 villages of three cultural traditions (Romanic, Germanic, and Walser). In valleys of Romanic villages more different parcel types tended to occur than in those of Germanic and Walser villages, suggesting that socio-economic differences among cultural traditions still play a role in shaping landscape diversity. Moreover, at the village level, higher man-made landscape diversity was associated with higher plant species richness. All observed changes in land use reduced the farmers' workload. Plant species richness was lower in fertilized than in unfertilized parcels and in abandoned compared with used parcels. Grazing slightly reduced species richness compared with mowing among unfertilized parcels, while in fertilized parcels it had a positive influence. The highest species diversity was found in mown unfertilized subalpine grasslands. Nevertheless, moderate grazing of former meadows can be a valuable alternative to abandonment. We conclude that observed changes in land use reduce plant species richness within parcels and at the landscape level. Financial incentives should not only maintain those types of land use that conserve high plant species diversity within grasslands, but should in addition promote a high diversity of land use types.

Keywords: biodiversity, grazing, mowing, species richness, conservation

Introduction

Human land use is one of the most important drivers of grassland biodiversity. In the cultural landscape of the Alps, one of the most plant species rich areas in Europe (Väre et al., 2003), human impacts are particularly pronounced. In Switzerland alpine grasslands have been influenced by humans for about 5000 years (Bätzing, 2003). Below timberline most grasslands are man-made and replace cleared forests. Current socio-economic changes are bringing about severe land use changes, mainly more intense use by using fertilizers or less intense use by abandonment, which may both negatively affect plant species richness. Therefore, land use changes receive most attention in the current debate on an adequate agricultural policy balancing conservation and socio-economic considerations. Less attention has been paid to their relative importance compared with topographic determinants. Moreover, despite the long history of grassland use in the Alps, the effect of cultural traditions on current biodiversity has not been studied, possibly due to the local scale of most studies.

In the Swiss Alps we distinguish three main old cultural traditions. The Romanic culture developed with the increase of the human population in Europe after 1800 BC, when settlements and land use were extended into Alpine valleys. It is characterized by a self-sufficient alpine economy practicing mixed farming with permanent compact villages at the valley bottoms, transient summer settlements at higher altitudes, and (sub)alpine pastures (Im Hof et al., 1986). Hereditary partitioning of parcels was frequent in the Romanic culture. The small-grained and frequently terraced parcel structure is still visible in many Romanic villages (Bätzing 2003). The Germanic culture developed after 600 AD, when Alemannic people from the North immigrated into the wetter northern and northeastern parts of the Alps. Germanic villages typically consisted of scattered single farm houses surrounded by fields and meadows, and the farmers practiced mainly dairy farming. Permanent fields for grain cultivation were rare, the farm was inherited as a whole by a son of the farmer, hereditary partitioning of parcels was uncommon and farms were usually larger than in Romanic villages (Bätzing, 2003). After 1200 AD, the Walser, Alemannic people from the Valais, migrated eastwards and settled at relatively high altitudes, as lower parts of the valleys were already occupied. As a consequence the Walser had to abandon cultivation of grain. Apart from farming they lived on trade and maintenance of alpine passes (Bätzing, 2003). Despite important changes since World War II, socio-economic differences are still remarkably pronounced among cultural traditions (Pfister, 2004). However, up to now it has not been studied whether these cultural traditions still affect current land use and biodiversity.

The effect of cultural traditions on plant species richness may be superimposed by effects of topography, climate, and soil conditions, which can vary considerably among regions in the Alps. Thus, landscapes may differ in the size of their regional species pools and thereby in the number of plant species which may potentially occur in grasslands (Pärtel et al., 1996). Moreover, at the landscape level, the number of different habitat types can affect species richness (Duelli, 1997).

The biodiversity of grasslands is influenced by geological, topographical, and climatic conditions. Particularly altitudinal gradients play a role (Rahbek, 1995; Theurillat et al., 2003). Human influence, represented by agricultural land use, also largely affects biodiversity and may exceed abiotic influence. Plant species diversity in man-made grasslands is up to three times higher than in the forests they are replacing (Zoller and Bischof, 1980) and may depend on the type and intensity of land use. In the agricultural landscape of the Alps, we find different kinds of agriculturally used grasslands. Pastures are grazed mainly by sheep and cattle, either only for a few weeks or during the whole summer. Meadows are cut between one and four times during one vegetation period. Some grasslands are fertilized and others are not.

Low-intensity farming promotes biodiversity, and more than 50% of the most highly valued biotopes in Europe are found in low-intensity farmland (Bignal and McCracken, 1996). However, for farmers low-intensity farming often means intensive human labor and a small yield (Bignal and McCracken, 1996). Therefore, a development towards abandonment of parcels used at low intensity far away from farms has been observed in recent decades, in Switzerland and in other European countries, which led to a dramatic decline in grassland area (Haefner and Günter, 1984; Olsson et al., 2000; Lindborg and Eriksson, 2004). On the other hand, the use of easily accessible parcels is intensified. More fertilizer is used, meadows are cut more often and pastures are grazed by more and larger cattle (Bätzing, 2003). However, although most of these issues have been addressed in local studies, larger-scale and comprehensive studies investigating the changes in land use and their consequences for biodiversity are missing in the Alps.

We comprehensively studied the effect of cultural traditions, altitude, and agricultural land use on plant species diversity in 216 parcels of land in 12 villages in the Swiss Alps. Each cultural tradition was represented by four villages, and the parcels were situated at three altitudinal levels. We studied grassland parcels that formerly had been mown or grazed, that today were mown, grazed or abandoned and that either were fertilized or unfertilized. We asked the following questions: (1) Does the likelihood of occurrence of a certain type of grassland parcel depend on cultural traditions, altitude, and land use? (2) Did land use

changes in the study parcels consistently indicate reduced labor for farmers? (3) Does the diversity of land use types affect plant species diversity in a village? (4) Does species diversity per parcel depend on cultural traditions, altitude, and land use, and which types of land use are associated with highest plant species richness?

Methods

Study area

We studied grasslands in 12 villages in the Swiss Alps, four of each of the three cultural traditions Romanic, Germanic, and Walser (Fig. 1). In this paper the term village refers to political municipalities that may comprise several smaller hamlets. The 12 villages are situated along an east-west gradient spanning 170 km, and each village belongs to a separate alpine valley. The villages were randomly selected with the restriction that their agricultural character had only changed modestly during the last 50 years, that they were not very touristy and did not have more than about 1500 inhabitants. Due to settlement history, the Germanic villages are located in the northern parts of the Alps and the Romanic and Walser villages in central and southern parts. The studied grasslands are mainly on siliceous bedrock forming brown podzolic soils, podzols, and rankers and in a few sites they are on calcareous bedrock forming gray-brown podzolic soils, and rendzinas (Frei et al., 1964).

Figure 1 Map of Switzerland with the 12 study villages and their cultural traditions.
● = Romanic, ■ = Germanic, ▲ = Walser

Regional species pools

As topography, climate, and soil conditions could vary considerably among villages, this could influence the number of species potentially inhabiting the grasslands of each village. The number of potential inhabitants is represented by the regional species pool (Pärtel et al., 1996). Therefore we used the regional species pools to explore potential effects of such topographic, climatic, and soil differences among villages. For each village we obtained the number of plant species of the regional species pool from "The Distribution Atlas of Pteridophytes and Phanerogams of Switzerland" (Welten and Sutter, 1982). For this atlas, species lists were compiled for almost 600 naturally defined sub-areas of about 100 km^2. Each area was classified as valley (from valley bottom to timberline) or mountain area (above timberline). For each of the 12 investigated villages we combined the species list of the concerned valley area with the surrounding mountain areas by using the software VEGEDAZ (Küchler 2004). To exclude species mainly occurring in wetlands or forests from this list we removed all species with ecological indicator values of 5 for moisture and 1 for light according to Landolt (1977). For each village we considered the number of remaining species on the corresponding list as size of the regional species pool. The sizes of the regional species pools were independent of the mapping area (between 154.8 and 324.6 km^2) and the altitudinal range of each pool (altitudinal differences between lowest and highest point of each area varied between 2019 and 3529 m).

Study design

In each village we chose parcels of land at three altitudinal levels, at the valley bottom (about 1000 m asl), at intermediate altitudes (in some regions called "Maiensäss", about 1500 m asl), and at the alp level (about 2000 m asl). We included grassland parcels that had been used in the same way for as long as it was known and parcels whose use had been changed five years ago or earlier. We looked for parcels which had formerly been mown or grazed, today were either mown or grazed or had been abandoned some years ago, and that were either additionally fertilized or unfertilized. Thus, for each village and altitudinal level we looked for parcels of 12 different combinations of land use, leading to a theoretical maximum of 432 parcels.

We selected the parcels in close collaboration with farmers in 2002. We took advantage of their knowledge of the land use history in their village and wanted to avoid selecting parcels not representative for the agricultural practice in each village. When it was impossible to find parcels whose type of land use had changed, we selected two parcels of the

concerned type of former land use to increase sample size. Altogether we selected 216 parcels, between 12 and 24 per village. The fertilized parcels were all fertilized with fertilizer produced on the farm, either with liquid manure or dung that had been collected in the stables. Only parcels that according to the farmers never had been fertilized were declared to be unfertilized. The unfertilized meadows were normally cut once a year while the fertilized meadows were cut between two and four times. Grazing intensity varied among years depending on the climatic conditions.

Vegetation records

In each parcel we randomly selected two 5 m x 5 m plots at a distance of 5 m. We classified each plot into three classes of structural heterogeneity (homogeneous, little heterogeneous, very heterogeneous). Additionally we recorded the coordinates and altitude of the parcels with a GPS, aspect as deviation from south in degrees, slope in degrees, and soil pH with a Hellige set (AVM Analyseverfahren, Freiburg, Germany).

In each plot we took a vegetation record according to a refined Braun-Blanquet (1951) method (for cover values above 15% we used categories of 10% difference: 16-25%, 26-35%, etc.). We visited each parcel once in 2002 or 2003, when the vegetation was best developed, usually shortly before the start of mowing or grazing. We estimated the ground cover percentage of all vascular plant species and counted the number of species *S* per record. For each record we calculated Shannon's index of diversity (Magurran, 1988) and Evenness as $E_{1/D} = \frac{1/D}{S}$. This evenness index is based on a diversity index derived from Simpson's index of dominance D ($D = \sum_i p_i^2$, $p_i =$ relative abundance of species *i*) and is independent of species richness (Smith and Wilson, 1996).

For each parcel we also calculated mean ground cover of the two plots per parcel for each species and obtained Shannon's index of diversity and Evenness also for these mean cover values. To measure the similarity of the species composition of the two records per parcel we calculated the Jaccard index as $C_J = \frac{j}{(a+b-j)}$, where *j* is the number of species found in both records per parcel, *a* is the number of species in record A, and *b* the number of species in record B (Magurran, 1988).

To estimate productivity in the plots, we harvested standing crop 4 cm above ground in a randomly selected sub-area of 0.5 m x 0.5 m. In already slightly grazed parcels we chose

areas in the plot where the vegetation had been untouched, provided that it could be considered to be representative for the vegetation of the whole parcel. In 16 cases we decided not to harvest at all. We air dried the samples in the field and afterwards dried them at 80°C for 24 hours before weighing them in the laboratory. To process species lists we used the software VEGEDAZ (Küchler, 2004).

Statistical analysis

Occurrence of land use types

To test which factors influence the occurrence of a specific land use type, we used a logistic regression model based on a binomial distribution, including the factors culture, village, altitude, fertilization, traditional land use, abandonment, and actual land use, and their two-way interactions. The villages were nested within cultures, and actual land use, which here refers to differences between mown and grazed grassland parcels, was nested within abandonment.

Diversity of land use types and species richness per village

We fitted an analysis of covariance (ANCOVA) model with sequential sums of squares to test whether the total number of species found in all investigated grassland parcels in a village depended on the number of combinations of altitude and land use types present. As covariables to test for landscape heterogeneity and to correct for differences in topography, we used the standard deviation of the aspects of all parcels in a village, mean slope, the standard deviation of the slopes, and the altitudinal range covered by the parcels per village.

To test whether confounding between number of land use types and investigated area per village could explain the number of species recorded per village, we compared it to the number of species expected based on the species-area curve calculated by Keel (1995) for vegetation records of species rich grasslands of the phytosociological Mesobromion community. Because our records span a much larger altitudinal gradient than those of Keel's study, we only used the records of the valley parcels of our study to compare observed and expected species numbers with a paired t-test. The species numbers found at the valley bottom of the 12 villages were on average 62 % higher than expected from the species-area curve ($P < 0.001$), indicating that differences between villages were not simply due to different numbers of study parcels.

Table 2 Summary of sequential sums of squares ANCOVA testing effects of abiotic variables, cultural traditions, and human land use on mean plant species richness per parcel among 216 grassland parcels in the Swiss Alps. Effects of culture and species pool were tested against remaining variation among villages. n.s. denotes values of $P > 0.1$. The non-significant interactions between culture and land use factors, between village and land use factors, and between altitude and land use factors were omitted from the table.

Source of variation	df	SS	*F*	*P*
Regional species pool	1	30.5	0.06	n.s.
Aspect	1	826.5	12.25	$P < 0.001$
Slope	1	3482.8	51.68	$P < 0.001$
pH	1	41.5	0.62	n.s.
$(pH)^2$	1	716.1	10.63	$P < 0.01$
Culture	2	82.2	0.08	n.s.
Village[Culture]	8	3985.2	7.39	$P < 0.001$
Altitude	1	1691.6	25.10	$P < 0.001$
$(Altitude)^2$	1	191.0	2.83	$P < 0.1$
Fertilization	1	4805.9	71.32	$P < 0.001$
Traditional land use	1	44.5	0.66	n.s.
Abandonment	1	2139.2	31.74	$P < 0.001$
Current land use [abandonment]	1	164.1	2.44	n.s.
Fertilization*traditional land use	1	226.3	3.36	n.s.
Fertilization*abandonment	1	7.2	0.11	n.s.
Fertilization*current land use[abandonment]	1	131.8	1.96	n.s.
Traditional land use*abandonment	1	147.2	2.18	n.s.
Culture*altitude	2	72.7	0.54	n.s.
Village[Culture]*altitude	9	3310.2	5.46	$P < 0.001$
Residuals	133	8962.6		

Species diversity at the parcel level

The basic unit of replication in our study is the parcel. To investigate effects of regional species pool, abiotic factors, cultural tradition, altitude, and land use combination on the measures of plant species diversity obtained per parcel and mean standing crop per parcel we used a hierarchical ANCOVA model with sequential sums of squares.

Regional species pools and the abiotic factors were used to account for regional differences in soil and climate which could affect species richness and diversity. To test for curvilinear relationships of pH and altitude we included the terms pH^2 and $altitude^2$ in the model. Effects of species pool and culture were tested against remaining variation among villages and of all other factors against variation due to remaining differences among parcels (Table 1).

Analyses of mean and total species numbers and the two different calculations of Shannon index and Evenness yielded qualitatively identical results. Therefore, we present results for mean species numbers and Shannon index and Evenness calculated based on mean cover values of the two records per parcel. To test whether pastures were more heterogeneous than meadows, we calculated a chi-square test using mean heterogeneity of two plots per parcel.

We performed all statistical analyses with the software R version 2.0.1 (R Development Core Team, 2004), except for the logistic regression for which we used GenStat (Version 6.1, GenStat Committee, VSN International, UK, 2002).

Results

Landscape diversity

Occurrence of land use types among cultural traditions

The highest diversity of land use types could be found in valleys of Romanic villages (interaction between culture and altitude, $P = 0.064$, Fig. 2). This trend suggests that cultural traditions are still playing a role in shaping our landscapes. The cultures differed at the valley bottom in the number of abandoned parcels as well as in the number of different types of abandoned parcels. In the four Romanic villages, altogether eight abandoned parcels of three different types were present, while in the Germanic and Walser villages there were only one and two formerly unfertilized meadows present, respectively.

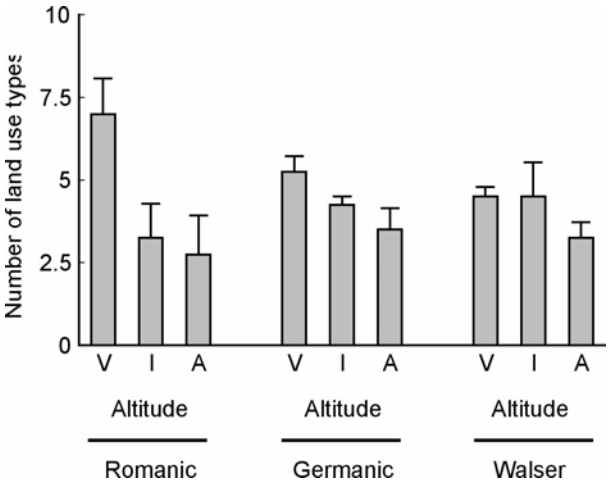

Figure 2 Mean number of land use types per cultural tradition and altitudinal level ± SE. V = Valley, I = Intermediate altitudes, A = Alp.

Table 2 Overview of study villages, their cultural traditions, numbers of land use by altitude combinations, total plant species numbers, and numbers of plant species in the regional species pool.

Village	Cultural tradition	No. of land use types	Total species number	No. of species in the regional species pool
Bedretto	Romanic	12	213	939
Braggio	Romanic	8	187	875
Ramosch	Romanic	21	284	1186
Trun	Romanic	10	193	1110
Gadmen	Germanic	13	272	877
Guttannen	Germanic	12	199	747
Linthal	Germanic	14	260	987
Unterschächen	Germanic	12	258	1093
Bosco Gurin	Walser	9	176	1021
Medels	Walser	14	242	862
Pany / Luzein	Walser	11	241	1129
Vals	Walser	11	218	853

The number of combinations of land use types and altitudinal levels found per village varied between 8 and 21, with a mean of 12.5 (Table 2). We found significantly more unfertilized than fertilized parcel types ($P < 0.001$), more grazed parcel types than mown ones ($P < 0.001$), and more different parcel types in valleys than at higher altitudes ($P < 0.01$). Fertilized parcels were more likely to be found in valleys than at the alp level (interaction between altitude and fertilization, $P < 0.05$).

Twenty-nine of the 126 formerly mown parcels now are grazed, while none of the 91 formerly grazed parcels now are mown. 23 formerly mown and 10 formerly grazed parcels, mostly unfertilized, have been left abandoned. Thus, all observed changes in land use had happened in the direction of reduced labor for the farmers.

Species richness per village

Independent of the cultural traditions, regional species pools varied between 747 and 1186 species (Table 1). We recorded 578 plant species in the 216 studied grassland parcels. Per village we found between 176 and 284 plant species in all study parcels, with a mean of 229 species (Table 2). We found highest species numbers in Germanic villages (247 ± 16 species, mean ± SE), followed by Romanic (219 ± 22 species) and Walser villages (219 ± 15 species). However, these differences were not statistically significant. The total number of species per village increased with the number of land use types occurring in a

village ($n = 12$, $P < 0.05$, Fig. 3) indicating that higher man-made diversity at the landscape level promotes higher plant species diversity. None of the topographic covariables that were used to test for effects of landscape heterogeneity explained significant variation in plant species diversity per village.

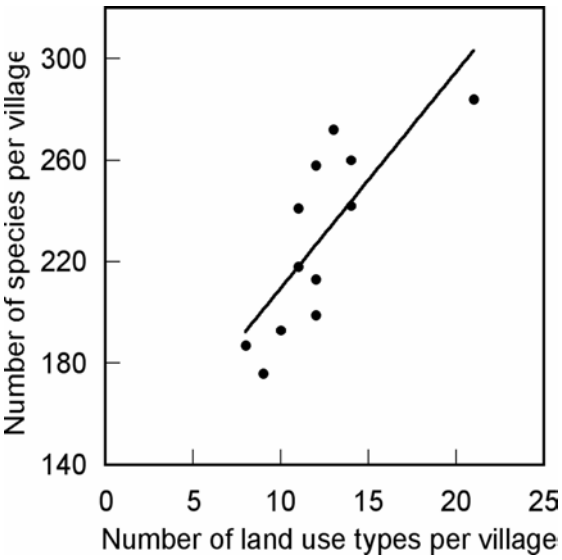

Figure 3 Relationship between the number of plant species recorded per village and the number of land use types.

Species diversity at the parcel level

We recorded between 15 and 84 plant species per 5 m x 5 m plot and between 20 and 101 plant species per parcel (2 plots of 5 m x 5 m). More species-rich parcels of land had a lower Jaccard index ($R = -0.20$, $P < 0.01$), indicating larger differences in species composition between the two plots in more species-rich parcels.

Neither cultural tradition nor the size of the regional species pools had a significant influence on species richness of parcels or any other of the investigated indices of plant species diversity per parcel. Species numbers increased with altitude ($P < 0.001$, Table 1), followed by a marginally significant decrease at the alp level (squared altitude effect, Table 1) The pattern of highest species richness at intermediate altitudes occurred in unfertilized and fertilized meadows and pastures (Table 3). More southern aspect, steeper slope and intermediate pH value were also associated with higher species richness (aspect, slope, and pH^2, Table 1).

Table 3 Plant species numbers per 5 m x 5 m (mean ± Standard error) of 216 grassland parcels of different land use at three altitudinal levels.

	Currently mown		Currently grazed	
	Unfertilized	Fertilized	Unfertilized	Fertilized
Valley	44.38 ± 2.06	28.57 ± 1.51	44.13 ± 1.93	34.21 ± 1.56
Intermediate	55.43 ± 3.26	31.23 ± 2.12	50.23 ± 2.72	36.62 ± 2.17
Alp	47.14 ± 4.15	37.0*	47.26 ± 2.75	34.7 ± 1.83

*only one parcel

Corresponding to our hypotheses, grassland biodiversity was strongly influenced by the type of land use (Fig. 4a). Mean species richness was lower in fertilized (32.5 species) than in unfertilized parcels (46.0 species, $P < 0.001$, Table 1) and it was lower in abandoned compared with currently used parcels ($P < 0.001$, Table 1), indicating negative diversity effects of fertilization and abandonment.

Among unfertilized, traditionally mown parcels, mean species richness was slightly higher in mown (49.1 species) than in recently grazed parcels (47.6 species, Fig. 4a), where it was still higher than in parcels that had always been grazed (47.0 species, Fig. 4a). In contrast to the case of unfertilized parcels, mean species richness was significantly higher in grazed (37.9 species) than in mown (29.7 species, a priori contrast, $P < 0.05$, Fig. 4a) among the fertilized, traditionally mown parcels. Qualitatively, we obtained the same results for Shannon index and Evenness, with the only exception of higher Evenness in mown than in grazed among the fertilized parcels.

Analyses of the Jaccard index showed smaller differences in species composition between two records in fertilized than in unfertilized parcels ($P < 0.05$), in traditionally mown than in traditionally grazed parcels ($P < 0.01$), and in used compared with abandoned parcels ($P < 0.001$). Pastures were structurally significantly more heterogeneous than meadows ($P < 0.001$).

Standing crop increased with fertilization ($P < 0.001$) and abandonment ($P < 0.001$). Among used parcels, fertilized meadows were more productive than fertilized pastures (Fig. 4b). Higher standing crop values (mean of two plots) per parcel were associated with lower mean species richness ($R = -0.25$, $P < 0.001$).

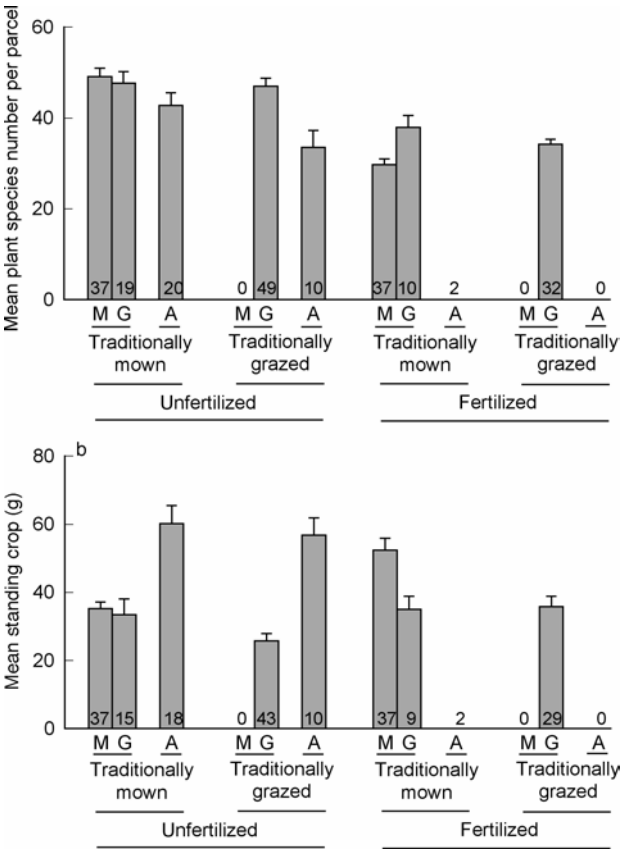

Figure 4 Relationship between a) mean (± SE) plant species number per parcel or b) mean (± SE) standing crop in two 0.5 m x 0.5 m areas per parcel and combination of fertilization, traditional land use, and current land use. M = Mown, G = Grazed, A = Abandoned. Figures in columns indicate numbers of replicate parcels.

Discussion

Cultural impact on landscape diversity

The trend towards a higher number of different land use types in valleys of Romanic villages suggests that cultural traditions in the Alps still influence landscape diversity. Because the land use decisions of Swiss farmers largely depend on subsidies based on an agricultural policy which does not differentiate among cultural traditions, the observed differences in land use diversity must indeed result from persisting socio-economic differences between cultures. Because plant species richness per village is the higher the more different land use types there are per village (Fig. 3), this implies that cultural traditions indirectly affect plant species diversity in a village.

In Romanic villages, where there are more different land use types in valleys than in villages of the other cultural traditions, land use structure at the valley bottom is likely to be more fine-grained, because of frequent hereditary partitioning of parcels and mixed farming in earlier decades (Bätzing, 2003). When parcels are partitioned over many generations, they can get too small for an efficient agricultural land use and are therefore likely to be abandoned, which could explain the higher frequency of abandoned parcels in the valleys of Romanic

compared to German and Walser villages. Although in a single parcel species richness and diversity can decrease after abandonment, a structurally diverse landscape including abandoned parcels, enhances overall species diversity (Zoller and Bischof, 1980). This holds true not only for plants but also for other groups of organisms, such as butterflies (Zoller and Bischof, 1980). While hereditary partitioning was also common in Walser villages, the smaller yield per area in these villages situated at high altitudes might have slowed down abandonment of agricultural land due to economic pressure.

In a recent socio-economic study comparing 140 Roman, German and Walser villages, 29 of 72 investigated socio-economic variables significantly differed among these cultural traditions (Pfister, 2004). When farms of the 12 study villages were compared, Walser farmers were found to manage parcels across a larger altitudinal range than Germanic and Romanic farmers, and to manage a larger proportion of particularly subsidized ecological compensation areas of generally high species diversity. Romanic farmers were found to manage the largest area per farm, and among Romanic farms the largest amount of organic farming was observed. Germanic farms, with low proportions of organic farming and with few ecological compensation areas, showed the most rapid decline in numbers over recent decades (Pfister 2004). While socio-economic factors of course do not influence plant species richness directly, they have an important influence on plant species richness via land-use diversity at the landscape level. The differences in ecological compensation areas and organic farming among cultural traditions are potential mechanisms for such land-use mediated diversity effects. We consider the observed cultural effects on land use diversity as especially noteworthy, as Swiss agricultural policy is rather promoting a homogenization of agricultural practices than taking cultural or regional peculiarities into account.

Diversity of plant species at the landscape level

We found more species in villages with more different land use types, and observed species numbers by far exceeded numbers expected from the species-area curve, indicating that land use diversity really played the decisive role. These results are in line with the mosaic concept, which proposes higher species numbers in landscapes with higher habitat diversity (Duelli, 1997). Higher landscape species richness associated with higher landscape diversity also can be found in the Loess Plateau in northern China comprising woodland, shrubland, grassland, farmland, and abandoned fields (Jiang et al., 2003). An increase in both landscape diversity and plant species numbers was found in semi-urban and urban compared with rural areas in Belgium (Honnay et al., 2003). The higher species diversity with higher landscape diversity

leads us to the very important conclusion, that agricultural policy should not only act at the level of single farms but also promote a diverse land use at the level of municipalities or even regions.

Direction of land use changes

We detected clear patterns in the direction of land use changes. While we found meadows that had been converted to pastures and formerly mown or grazed parcels that had been left abandoned, there were no pastures that had been converted to meadows. Pastures with a certain slope very often show remarkable horizontal cow tracks. They are therefore not suitable for mowing due to their structural heterogeneity, which may impede their conversion to meadows. Nevertheless, our findings clearly show the important socio-economic trend that all observed changes in land use reduced the workload of the farmers (Bätzing, 2003).

Relationship between altitude and plant species diversity at the parcel level

Plant species richness was highest at intermediate altitudes. As Rahbek (1995) showed in his review of 97 papers, different altitude-species richness patterns can be observed. Among plants, a hump-shaped pattern, as in our study, appears most common. Most likely explanation for the highest plant species diversity observed at intermediate altitudes appears to be the overlap of subalpine and alpine species pools (Grytnes, 2003).

Effects of fertilization and abandonment on plant species diversity at the parcel level

Plant species richness was significantly reduced in fertilized parcels. Various experimental studies show reduced species diversity in grasslands after nutrient addition (e.g., Foster and Gross, 1998; Jacquemyn et al., 2003; Baer et al., 2004). In calcareous fen grasslands, generalist species increase after experimental fertilization, and obtained results suggest that species composition would change and species richness would decrease over longer time periods (Pauli et al., 2002). In the Italian and Austrian Alps, species numbers in mountain meadows decreased when land use was intensified and parcels were fertilized (Tasser and Tappeiner, 2002).

Species richness was also clearly reduced in abandoned compared with used parcels as it has been observed in other studies, (e.g., Kahmen and Poschlod, 2004). Due to missing disturbance events a thick layer of dead plant material covers the ground of abandoned parcels. There, tall forbs and clonal and highly competitive grasses can show high growth rates. Therefore, competition increases and light availability decreases, leading to a higher

mortality of sub-canopy plants (Jacquemyn et al., 2003). In the long run there is a shift in vegetation composition towards dwarf shrub or forest communities (Tasser and Tappeiner, 2002). Of course, abandonment is not only a problem concerning biodiversity loss, but, depending on aspect, slope, and soil depth, it can also enhance the probability of landslides (Tasser et al., 2003), which may endanger roads and settlements.

Effects of mowing and grazing on plant species diversity at the parcel level

We found highest plant species richness in unfertilized meadows. However, the difference to unfertilized pastures that formerly had been mown was small. In contrast, species numbers in unfertilized pastures that had always been grazed were lower than in the pastures that were previously mown. This indicates that in the long run species richness of meadows will decrease when they are converted to pastures (Fischer and Wipf, 2002).

In contrast to unfertilized grassland parcels, grazing enhanced species richness among the fertilized parcels. A comparison of 30 studies showed that such a reversed impact of grazing in nutrient-poor versus nutrient-rich ecosystems can be observed among different types of habitats (Proulx and Mazumder, 1998). Such an interaction of productivity with the grazing effect in mountain habitats, as suggested by a review of Austrheim and Eriksson (2001), is supported by our results. Pastures are structurally more heterogeneous, probably because naturally homogeneous grassland parcels were rather selected to be mown than to be grazed, but also due to cow prints and more heterogeneously distributed nutrients and uneven seed input by feces. This heterogeneity offers a higher diversity of available ecological niches (Duelli and Obrist, 2003) and may promote species richness at high nutrient levels. In nutrient-poor habitats, however, intensive grazing reduces species richness because plants have a limited capacity for regrowth (Proulx and Mazumder, 1998). Although grazing reduces species richness compared with mowing, this loss of biodiversity is not as severe as after abandonment. Therefore, grazing can help to conserve at least part of the species richness while simultaneously reducing the workload for the farmer. Traditionally, however, many parcels that were too steep to be grazed by cattle had been mown, and nowadays their plant species richness is threatened by abandonment.

The analyses using Shannon index and Evenness largely confirmed our findings on species richness. The only exception was that Evenness was highest in mown fertilized grasslands, whereas species richness was highest in unfertilized ones. This is probably due to the few but rather abundant, highly competitive species in these grassland parcels and to the

absence of cow prints, which could relieve competition pressure and serve as safe sites for recruitment of rarer species.

Relationship between species diversity and standing crop

Our data confirmed a conflict between the conservation goal of high plant species richness and the agricultural goal of high productivity. In fertilized meadows with the highest standing crop, only half as many species occurred than in unfertilized meadows. Standing crop of fertilized pastures was not much higher than that of unfertilized pastures, but their species richness was much lower. Because this implies little yield gain from fertilization but a severe loss of biodiversity, pastures should not be fertilized at all. Admittedly, there will always be some fertilized pastures, because farmers have to dispose of the manure collected in the stables, and when there are no meadows in a reasonable distance to the stable, the manure is spread on the surrounding pastures. Beyond that, however, pastures should not be fertilized.

Conclusions

Our comprehensive study across 12 villages along a 170 km east-west gradient suggests that cultural traditions still affect man-made landscape diversity. This cultural footprint on the number of land use types can be explained by still persisting socio-economic differences among cultural traditions. High land use diversity in turn was related to high plant species richness in villages and thereby is important to conserve a high plant species diversity.

Observed land use changes reduce the workload of the farmers, but at the same time decrease plant species diversity. Unfortunately, unfertilized meadows with the highest species richness require much labor of the farmer and provide little yield. While the conversion of such unfertilized meadows to extensively grazed pastures could be a valuable alternative to abandonment, in the long run it will result in a reduction of species numbers. Overall, financial incentives should not only maintain those types of land use that conserve high plant species diversity, but should in addition promote a high diversity of land use types.

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2

Leaf herbivory and fungal pathogen infection in grasslands in the Swiss Alps

(submitted)

Anne Weyand, Katrin Maurer, Jürg Stöcklin, Markus Fischer

Summary

1. In the cultural landscape of the Alps, grassland contributes to high biodiversity not only by its plant species richness, but also by the diversity of biological interactions between plants, herbivores, and pathogens.
2. We recorded the leaf area damaged by ten types of herbivory and five types of fungal pathogen infection on 12'054 plant leaves of legumes, other forbs, and graminoids collected in 215 grassland parcels of different land use and altitude in the Swiss Alps when vegetation was best developed.
3. We asked how the extent of leaf damage by herbivory and fungal pathogen infection, and their diversity, are affected by plant functional group, land use, and altitude, and whether extent and diversity of leaf damage are related to each other, to plant species richness, and to standing crop.
4. With 83% of all leaves infested, herbivory and fungal pathogen infection were ubiquitous. However, only 2.7% leaf area was damaged by herbivory and 1.2% by fungal pathogens. Damage by herbivory was highest on legumes, and damage by fungal pathogens was highest on graminoids. More leaf damage by herbivory occurred in traditionally mown sites and at lower altitudes, while damage by fungal pathogen infection was independent of land use and altitude. Most types of herbivory were found on legumes and on leaves from fertilised sites, whereas the diversity of fungal pathogen types was highest on graminoids and in unfertilised sites. Larger standing crop was associated with higher leaf damage and diversity of herbivory types per leaf. In parcels with higher number of plant species per parcel the diversity of herbivory types was lower at the leaf level, but tended to be higher at the parcel level.
5. Our results highlight the omnipresence of plant-herbivore and plant-pathogen interactions. Moreover, they indicate neither a conflict between conservation goals for different taxa, nor a conflict between the conservation of biological interactions of plants and agricultural goals in the Alps. Therefore, we suggest maintaining the high diversity of mountain grassland by diverse low-intensity farming.

Key words: agricultural land use; biodiversity; biological interactions; conservation; forbs; fungi; grasses; herbivores; legumes; plant functional groups

Introduction

Meadows and pastures are an important element of the cultural landscape of the Alps and are famous for their characteristic and diverse flora and fauna (Ellenberg, 1996; Zoller & Bischof, 1980). The various landscapes of the Alps have been shaped by humans and their cattle over thousands of years (Ellenberg, 1996) and they are among the most plant species rich areas in Europe (Väre et al., 2003). Different cultural traditions (Germanic, Romanic, and Walser) with their specific farming practices have contributed to the high diversity of the alpine landscapes (Bätzing 1991). However, in the Alps land use is undergoing major changes (Bätzing, 2003) where both abandonment and intensification of land use constitute major threats to alpine plant species diversity (Fischer & Wipf, 2002; Olsson et al., 2000).

Biodiversity consists of diversity at several levels of biological integration (communities, species, genes), and includes biological interactions such as pollination, herbivory, and fungal disease (Primack, 1993). In addition to land use, biodiversity is also affected by geological, topographic, and climatic conditions. In the Alps, the altitudinal gradient is particularly important (Rahbek, 1995; Theurillat et al., 2003). Efforts to protect the diversity of alpine landscapes should be based on a comprehensive knowledge of biodiversity at all levels and spatial scales. However, while plant species richness is comparatively well studied in grasslands in the Alps, information about the diversity of herbivores and plant pathogens is very scarce.

Conflicts between different conservation goals arise if high diversity of herbivores or plant pathogens is associated with low diversity of the other group, or of plants. Moreover, if high diversity of herbivores and pathogens were associated with substantial plant biomass losses, their conservation would conflict with the agricultural goal of high biomass production. Currently, the relationships between plant species richness, diversity of herbivory and pathogens, and standing crop are not known for mountain grassland.

Several studies with herbivores and pathogens in grassland at lower altitudes suggest that degree and diversity of herbivory and pathogen infection are affected by land use (Kruess & Tscharntke, 2002; Wettstein & Schmid, 1999), site productivity (Mulder et al., 2002; Siemann, 1998), and plant species diversity (Knops et al., 1999; Mitchell et al., 2002). Moreover, altitudinal effects on the extent of herbivory have been reported for molluscs (Scheidel & Bruelheide, 2001). In wetlands in the pre-Alps, the diversity and abundance of grasshoppers and butterflies was negatively affected by management and declined with altitude (Wettstein and Schmid 1999). However, a comprehensive study of degree and diversity of herbivory and pathogen infection is missing for grassland at higher altitudes.

Different plant functional groups are likely to be differently affected by herbivores and pathogens (Symstad et al., 2000). Due to their high nitrogen contents, legumes are likely to be preferred by herbivores (Mattson, 1980). Pathogens are more likely and more abundant in larger host populations (Mitchell et al., 2002). Therefore, due to their usually high abundances, graminoids may be more likely to be infected by fungal pathogens than forbs or legumes are.

We measured the amount of leaf damage by herbivory and by fungal pathogen infection at three altitudinal levels around each of 12 villages across the Swiss Alps as an indicator for the intensity of plant-herbivore and plant-pathogen interactions in mountain grassland. We measured leaf damage once, whenever possible at the time of maximum standing crop, when the level of damage is most relevant for farmers. To measure diversity, we classified leaf damage according to different types of herbivory and fungal pathogen infection. This indirect approach to measure biological interactions allowed us to quantitatively assess leaf damage by different types of herbivory and fungal pathogen infection for 215 grassland parcels.

For scoring leaf damage by different herbivory types we followed Crawley (1983) who classified leaf damage by herbivores according to feeding habit. Because single damage types can be caused by several taxa, it is not possible to identify the species that caused a particular damage type. Nevertheless, Crawley's classification of damage types of herbivores allowed us to assess the diversity of herbivory. For fungal pathogen infection, the situation is more difficult. There is no such classification of leaf damage by different types of fungal pathogens. Therefore, for fungal pathogens we used broad taxonomic categories that could be distinguished with a binocular microscope.

We studied the degree of damage by different types of herbivory and fungal pathogen infection of leaves of 215 grassland parcels of different land uses at different altitudes in the Swiss Alps. To account for potential regional and cultural differences, these grassland parcels represented four villages of each of the three main cultural traditions in the Swiss Alps, Romanic, Germanic, and Walser. Our main questions were: (1) How large is the damage of plant leaves by herbivory and fungal pathogen infection, and does the extent of leaf damage depend on plant functional group, land use, and altitude? (2) Which, and how many, different types of herbivory and fungal pathogen infection are present on leaves of different origin, and do number and composition of types depend on plant functional group, land use, and altitude? (3) How are leaf damage and the diversity of types of herbivory and fungal pathogen infection

related to each other, to plant species richness, and to the amount of standing crop per grassland parcel?

Materials and methods

Study sites

We studied grassland in 12 villages in the Swiss Alps, four of each of the three cultural traditions (named after the original settling tribes) Romanic, Germanic, and Walser (Chapter 1). Each village belongs to a separate alpine valley, and the 12 villages are located along an east-west gradient of about 170 km. We selected villages from those whose agriculture has only changed modestly during the last 50 years, that are not very touristy, and that have less than 1500 inhabitants.

At three altitudinal levels per village (valley at about 1000 m asl, intermediate altitudes about 1500 m asl, and alp at about 2000 m asl), we selected parcels of land representing up to 12 different land use combinations (Chapter 1). Parcels were fertilised or unfertilised, had traditionally been mown or grazed, and were currently mown, grazed, or abandoned. In total, we selected 216 grassland parcels, ranging from 12 to 24 per village.

Leaf sampling

We had taken vegetation records in two 5 m x 5 m plots separated by about 5 m in each grassland parcel in summer 2002 (69 parcels) and summer 2003 (147 parcels) to assess plant species composition and diversity (Chapter 1). As close as logistically possible, we did this when the vegetation was best developed, shortly before managed parcels were mown or grazed. At this time, leaf damage is most relevant for farmers. Additionally, in each plot we harvested biomass 4 cm above ground in a randomly selected area of 0.5 m x 0.5 m to assess standing crop. At the same time, in 215 of the 216 parcels we collected one leaf of each of ten plants of the three functional plant groups graminoids (Poaceae, *Carex,* and *Luzula*), legumes, and non-legume forbs (except for thistles and orchids). In each plot, we collected leaves of plants closest to positions defined by a walking grid. This procedure resulted in a sample of 30 randomly selected leaves per plot, i.e. 60 leaves per parcel, except for parcels without or with only few legume plants. We transported the leaves in plastic bags, pressed them in plant presses for up to five days, and dried them in a drying oven at 40°C for at least two days.

Scoring of herbivory and fungal pathogen infection

In winter 2003/2004, we visually screened all 12'054 leaves for damage by herbivory and fungal pathogen infection with a binocular microscope (total magnification 40x). To classify different types of leaf damage, we distinguished ten categories of infestation by herbivory and five categories of infestation by fungal pathogen infection (Table 1).

Table 1 Ten types of leaf damage by herbivory and five types of leaf damage by fungal pathogens and their characteristics scored on 12'054 leaves of plants from 215 grassland parcels in the Swiss Alps.

Category	**Characteristics**
Herbivory	
Clipping	Damage of leaf margins; usually sharp edges
Holing	Damage within leaf; continuous hole in the leaf
Windowing	Damage within leaf, but with remaining single cell layer
Rasping	Removal of single cell layers from surface; often white short dashes
Mining	Continuous mine in the leaf, visible from both sides
Skeletonising	Damage by continuous hole along the leaf veins
Spinning	Cocoons or cocoon-like structures
Puncturing	Fine punctured stitch-like pattern
Eggs	Mostly accumulated, but also single ones
Larvae/pupae	Mostly single
Fungal pathogens	
Rust fungus	Greyish sporangia with brown rusty spores, crumb-like on leaf surface or veins
Hyphomycetes	Small, black, cloud-like structures on leaf surface
Powdery mildew	Patches of fine white mycelium, mostly on upper leaf surface
Ascomycetes	Patches with pustule-like structure in the centre
Unknown Fungi	Undefined damage (patches with parchment-like inner part, holes with red margin, mine visible from only one side, other concentric coloured patches with light centre/dark margin)

For herbivory, we distinguished between clipping, holing, rasping, mining, skeletonising, and spinning according to Crawley (1983). Additionally, we considered the categories windowing, puncturing, eggs, and larvae/pupae. We considered eggs and larvae/pupae after realising their frequent presence and large numbers on the leaves. We are aware that scored eggs and larvae/pupae would have developed into one of the other categories. Nevertheless, introducing the classes of eggs and larvae/pupae were not only due to practicability, because the simultaneous occurrence of different stages of development also constitutes diversity.

We scored fungal pathogen infection according to taxonomical categories and distinguished between rust fungi, powdery mildew, hyphomycetes, and ascomycetes. Because it was not possible to unambiguously classify all infestations by fungal pathogens, we also introduced the category of unknown fungi, which may however to some degree include infection by the groups mentioned above. Finally, we classified any leaf damage as unknown, which could not be ascribed to any of these categories. We did not include such unknown damage into the analyses.

For each leaf, we estimated the leaf area damaged by herbivory and fungal pathogen infection separately in percent. Then, we recorded the presence and absence of all categories of leaf damage. Additionally, we noted the category that caused the largest area of leaf damage, both for herbivory and fungal pathogen infection. To estimate the proportion of damage per leaf we compared the leaves with leaf templates with known proportions of damaged area. For damage amounting to less than one percent, we used the value 0.5 for the calculations.

Data analysis

We included all 12’054 leaves and assessed damage by herbivory and by fungal pathogen infection per leaf, per functional group and parcel, and per parcel. We analysed the effects of land use and altitude on damaged leaf area and diversity of types of herbivory and fungal pathogen infection with analyses of variance (ANOVA) using a hierarchical mixed model including the factors culture, village, altitude, fertilisation, abandonment, current land use, traditional land use, parcel and all interactions (Table 2). Villages were nested within cultures, and current land use was nested within abandonment, because in our study current land use is meant to refer to currently mown and grazed grassland parcels. To analyse differences between functional groups, we

Table 2 ANOVA model of leaf damage by, and diversity of types of, herbivory and fungal pathogen infection on 12'054 leaves of plants from 215 grassland parcels of different altitude and land use in 12 villages of 3 different cultural traditions in the Swiss Alps.

Source of variation	Mean Squares	Variance ratios (F-values)
Culture	ms_{cult}	ms_{cult} / ms_{vill}
Village	ms_{vill}	$ms_{vill} / ms_{residual}$
Altitude	ms_{alt}	$ms_{alt} / ms_{residual}$
Fertilisation	ms_{fert}	$ms_{fert} / ms_{residual}$
Traditional land use	ms_{trad}	$ms_{trad} / ms_{residual}$
Abandonment	ms_{aband}	ms_{aband} / ms_{curr}
Current land use	ms_{curr}	$ms_{curr} / ms_{residual}$
Traditional land use x Fertilisation	$ms_{trad\ x\ fert}$	$ms_{trad\ x\ fert} / ms_{residual}$
Abandonment x Fertilisation	$ms_{aband\ x\ fert}$	$ms_{aband\ x\ fert} / ms_{curr\ x\ fert}$
Current land use x Fertilisation	$ms_{curr\ x\ fert}$	$ms_{curr\ x\ fert} / ms_{residual}$
Abandonment x Traditional land use	$ms_{aband\ x\ trad}$	$ms_{aband\ x\ trad} / ms_{residual}$
Culture x Altitude	$ms_{cult\ x\ alt}$	$ms_{cult\ x\ alt} / ms_{vill\ x\ alt}$
Village x Altitude	$ms_{vill\ x\ alt}$	$ms_{vill\ x\ alt} / ms_{residual}$
Culture x Fertilisation	$ms_{cult\ x\ fert}$	$ms_{cult\ x\ fert} / ms_{vill\ x\ fert}$
Culture x Traditional land use	$ms_{cult\ x\ trad}$	$ms_{cult\ x\ trad} / ms_{vill\ x\ trad}$
Culture x Abandonment	$ms_{cult\ x\ aband}$	$ms_{cult\ x\ aband} / ms_{vill\ x\ aband}$
Culture x Current land use	$ms_{cult\ x\ curr}$	$ms_{cult\ x\ curr} / ms_{vill\ x\ curr}$
Village x Fertilisation	$ms_{vill\ x\ fert}$	$ms_{vill\ x\ fert} / ms_{residual}$
Village x Traditional land use	$ms_{vill\ x\ trad}$	$ms_{vill\ x\ trad} / ms_{residual}$
Village x Abandonment	$ms_{vill\ x\ aband}$	$ms_{vill\ x\ aband} / ms_{residual}$
Village x Current land use	$ms_{vill\ x\ curr}$	$ms_{vill\ x\ curr} / ms_{residual}$
Fertilisation x Altitude	$ms_{fert\ x\ alt}$	$ms_{fert\ x\ alt} / ms_{residual}$
Traditional land use x Altitude	$ms_{trad\ x\ alt}$	$ms_{trad\ x\ alt} / ms_{residual}$
Abandonment x Altitude	$ms_{aband\ x\ alt}$	$ms_{aband\ x\ alt} / ms_{curr\ x\ alt}$
Current land use x Altitude	$ms_{curr\ x\ alt}$	$ms_{curr\ x\ alt} / ms_{residual}$
Culture x Altitude x Fertilisation	$ms_{cult\ x\ alt\ x\ fert}$	$ms_{cult\ x\ alt\ fert} / ms_{vill\ x\ alt\ x\ fert}$
Culture x Altitude x Traditional land use	$ms_{cult\ x\ alt\ x\ trad}$	$ms_{cult\ x\ alt\ x\ trad} / ms_{vill\ x\ alt\ x\ trad}$
Culture x Altitude x Abandonment	$ms_{cult\ x\ alt\ x\ aband}$	$ms_{cult\ x\ alt\ x\ aband} / ms_{vill\ x\ alt\ x\ aband}$
Culture x Altitude x Current land use	$ms_{cult\ x\ alt\ x\ curr}$	$ms_{cult\ x\ alt\ x\ curr} / ms_{vill\ x\ alt\ x\ curr}$
Village x Altitude x Fertilisation	$ms_{vill\ x\ alt\ x\ fert}$	$ms_{vill\ x\ alt\ x\ fert} / ms_{residual}$
Village x Altitude x Traditional land use	$ms_{vill\ x\ alt\ x\ trad}$	$ms_{vill\ x\ alt\ x\ trad} / ms_{residual}$
Village x Altitude x Abandonment	$ms_{vill\ x\ alt\ x\ curr}$	$ms_{vill\ x\ alt\ x\ aband} / ms_{residual}$
Village x Altitude x Current land use	$ms_{vill\ x\ alt\ x\ aband}$	$ms_{vill\ x\ alt\ x\ curr} / ms_{residual}$
Residual	$ms_{residual}$	

added the factor functional group and all its interactions to the model. In all analyses, we treated village and parcel as random factors. Aspect and slope of a parcel, and yearly differences could affect herbivores and fungal pathogens. However, including aspect, slope, and year of leaf harvest as covariates turned out not to change levels of significance. Therefore, we present results of the model without covariates.

To analyse differences in the composition of damage types between parcels, first, we conducted a principal component analysis (PCA) of the occurrence of different categories of herbivory and fungal pathogen infection per leaf. Then, to test for effects of altitude and land use on the composition of herbivory and fungal pathogen diversity we did an ANOVA of the four principal components with the same model as mentioned above.

Results

Leaf damage by herbivory and fungal pathogen infection

82.8% of the 12'054 leaves were damaged by at least one category of herbivory or fungal pathogen infection. The most frequent categories of herbivory were, in declining order, clipping, holing, rasping, and windowing (Table 3). Moreover, eggs were present on 49% of all leaves and dominant on 25.1% of them, indicating that damage by and number of occurring herbivory types would presumably increase after egg development. For fungal pathogen infection, the most frequent categories, in declining order, were rust fungi (3.5%), hyphomycetes (2.1%), powdery mildew (1.2%), and ascomycetes (0.4%). However, these numbers were small compared to the 18.9% of leaves with unknown fungi (Table 3). 58.7% of the leaves were infested by herbivory only, 6.0% by fungal pathogens only, and 18.0% by both.

We recorded 2.7 ± 0.13% (mean ± SE) leaf damage by herbivory, and 1.2 ± 0.09% by fungal pathogen infection. Parcels with higher damage of leaf area by herbivory also had higher damage by fungal pathogen infection ($N = 215$, $R = 0.18$, $p < 0.01$, Fig. 1A). Herbivory was most pronounced on legumes (4.5 ± 0.3% damage per leaf), followed by non-legume forbs (2.6 ± 0.1%), and graminoids (1.3 ± 0.1%, Fig. 2A). In contrast, fungal pathogen infection was most pronounced on graminoids (1.5 ± 0.1%), followed by non-legume forbs (1.2 ± 0.1%), and legumes (0.7 ± 0.1%, Fig. 2A).

Table 3 Percentage of leaves of legumes, non-legume forbs, and graminoids damaged by 10 different herbivory types, by 5 different fungal pathogen types, and by unknown causes in 215 grassland parcels in the Swiss Alps. Also included are the percentage of each damage category present per parcel, and the percentage of leaves for which the particular category was the dominant cause of damage.

Type of leaf damage	**Present in x % of**					**Dominant in x% leaves**
Herbivory	**Leaves of legumes**	**Leaves of non-legume forbs**	**Leaves of graminoids**	**Leaves of all groups**	**Parcels**	
Clipping	38.29	23.12	8.09	22.43	100.0	16.9
Holing	38.23	20.84	4.15	20.24	99.5	10.0
Windowing	28.34	15.59	8.97	17.11	99.5	7.5
Rasping	27.45	12.63	20.56	19.86	94.9	14.3
Puncturing	2.51	2.56	0.24	1.73	45.6	0.4
Skeletonising	2.32	0.19	0.47	0.93	28.8	0.3
Mining	0.94	0.52	1.12	0.85	29.8	0.4
Spinning	0.52	1.00	0.14	0.56	16.3	0.2
Eggs	54.74	53.10	39.95	49.00	100.0	25.1
Larvae/pupae	2.48	5.91	1.83	3.45	74.4	1.5
Fungal pathogens						
Rust fungi	14.44	19.23	22.43	18.91	65.1	3.1
Hyphomycetes	4.05	2.49	4.11	3.53	43.7	1.1
Powdery mildew	2.34	1.38	2.52	2.07	23.3	1.1
Ascomycetes	1.71	1.33	0.57	1.18	16.7	0.4
Unknown Fungi	0.69	0.52	0.14	0.44	97.7	18.3
Unknown	8.3	9.2	5.7	23.3	100.0	-

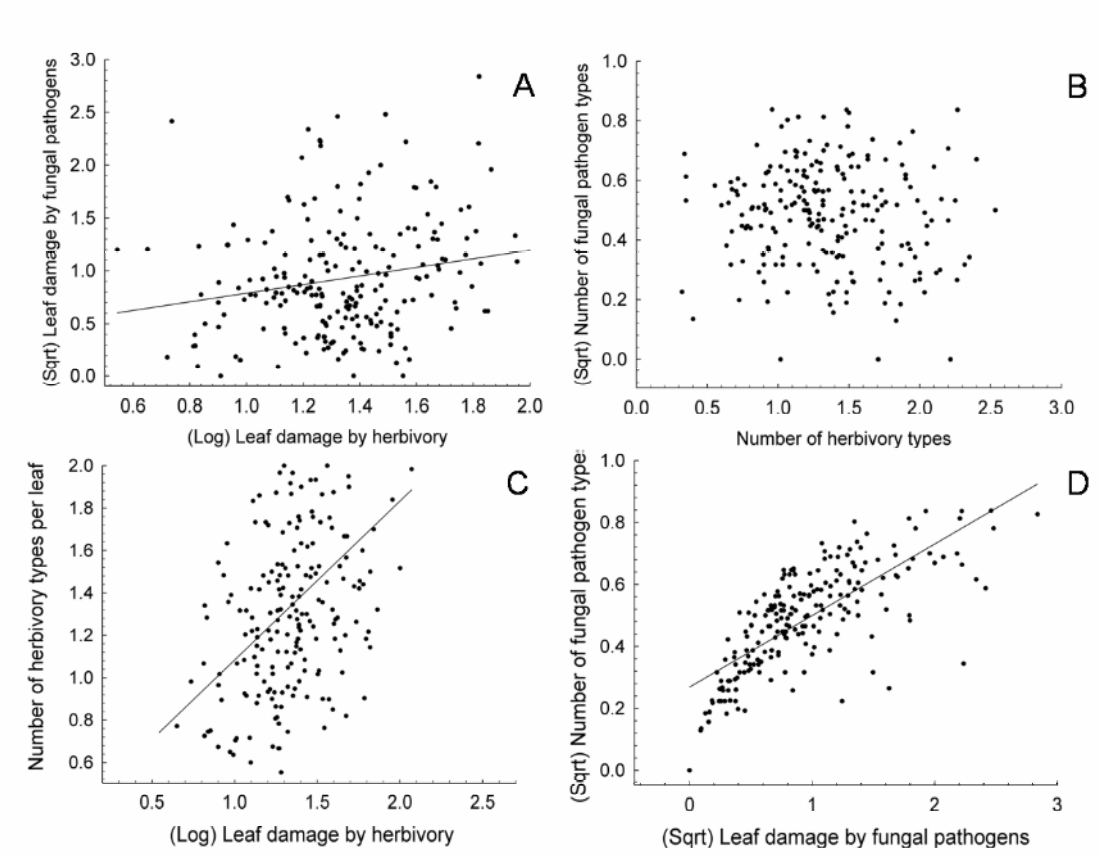

Figure 1 Relationship between A) leaf damage by herbivory and by fungal pathogen infection, B) diversity of types of herbivory and fungal pathogen infection, C) leaf damage and the diversity of types of herbivory and D) leaf damage and diversity of types of fungal pathogen infection on leaves from 215 grassland parcels in the Swiss Alps. Sqrt denotes square root transformation, log denotes logarithm to the base of ten.

The lower the altitude, the more leaf area was damaged by herbivory (valley 3.3 ± 0.2%, intermediate altitudes 2.6 ± 0.2%, alp 2.0 ± 0.2%; N = 215, $F_{1,98} = 34.1$, $p < 0.001$). The damaged leaf area of legumes was higher in the valley than at intermediate altitudes, where it was higher than at the alp level (N = 638, $F_{2,199} = 14.1$, $p < 0.001$, Fig. 3A). On graminoids, damage was also higher in the valley grassland, but did not differ between grassland at intermediate altitudes and at the alp level. Leaf damage of forbs was independent of altitude. Moreover, altitude did not affect damage by fungal pathogen infection.

In traditionally mown grassland, more leaf area was damaged by herbivory on legumes and forbs than in those that had always been grazed, whereas there was no such effect for graminoids (N = 638, $F_{2,199} = 5.6$, $p < 0.01$, Fig. 3B). Moreover, land use did not affect leaf damage by fungal pathogens.

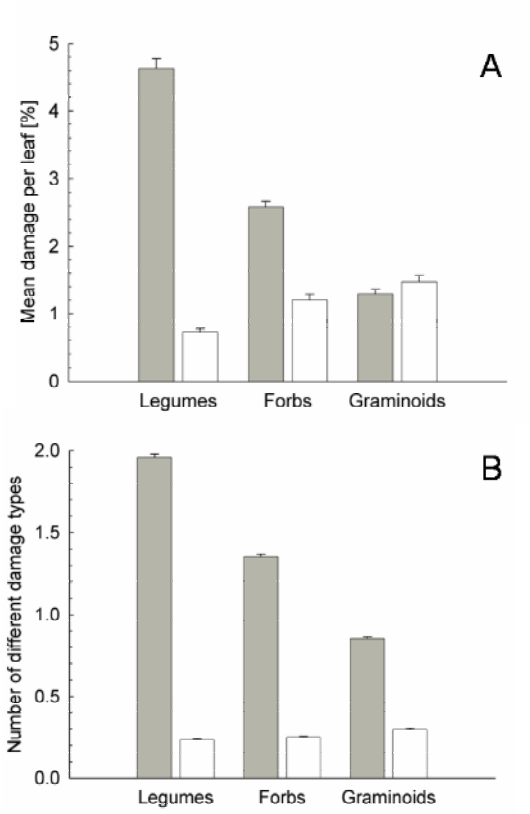

Figure 2 A) Mean damage per leaf by herbivory (shaded) and fungal pathogen infection (open), and B) mean number of different damage types by herbivory (shaded) and fungal pathogen infection (open) recorded on leaves of the three functional groups (legumes, non-legume forbs, graminoids) from 215 grassland parcels in the Swiss Alps. Error bars denote 1 standard error.

Diversity of types of herbivory

Per leaf, we recorded 1.36 ± 0.01 of the ten types of herbivory. Per parcel, we recorded 6.89 ± 0.07 types of herbivory. The number of herbivory types per leaf was higher in parcels with higher average leaf damage by herbivory (N = 215, R = 0.40, $p < 0.001$, Fig. 1C).

More herbivory types occurred on legumes (1.90 ± 0.05 types per leaf) than on non-legume forbs (1.35 ± 0.03), and graminoids (0.86 ± 0.03, Fig. 2B). The lower the altitude, the more types of herbivory occurred per leaf (valley 1.53 ± 0.05 types per leaf, intermediate altitudes 1.40 ± 0.04, alp 1.01 ± 0.04; N = 215, $F_{1,98} = 137.8$, $p < 0.001$). This was more pronounced for legumes than it was for graminoids and non-legume forbs (functional group by altitude interaction; N = 638, $F_{2,199} = 3.2$, $p < 0.05$, Fig. 4A).

More types of herbivory occurred in traditionally mown grassland parcels (1.45 ± 0.04 types per leaf) than in grazed ones (1.20 ± 0.04, N = 215, $F_{1,98} = 18.3$, $p < 0.001$, Fig. 4B). Moreover, more types of herbivory occurred in fertilised (1.62 types per leaf ± 0.03) than in unfertilised grassland (1.18 ± 0.05, N = 215, $F_{1,98} = 42.4$, $p < 0.001$, Fig. 4C). The differences in the numbers of types of herbivory per leaf which were highest on legumes, intermediate on non-legume forbs, and lowest on graminoids, were more pronounced in traditionally mown

grassland parcels than in traditionally grazed ones (functional group by traditional land use interaction, N = 638, $F_{2,199} = 8.8$, $p < 0.001$, Fig. 4B).

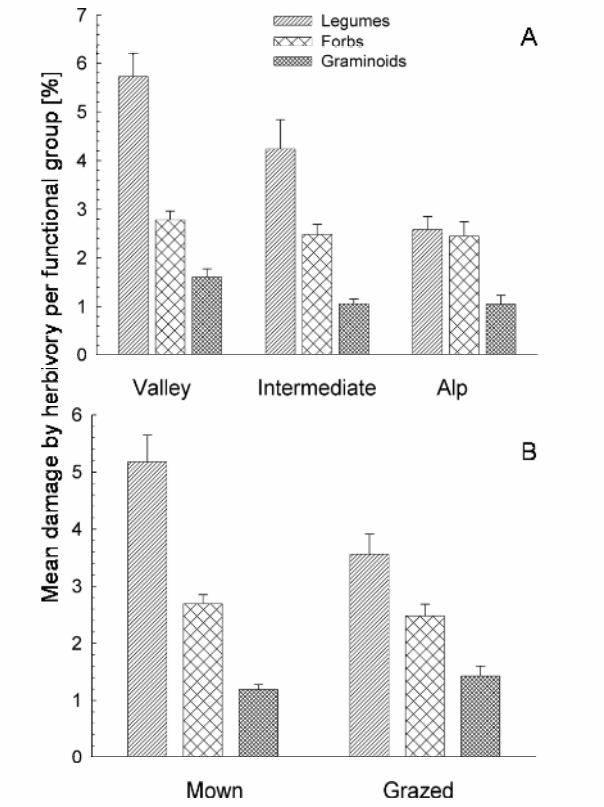

Figure 3 The relationship between mean leaf damage by herbivory per functional group (legumes, non-legume forbs, graminoids) in 215 grassland parcels in the Swiss Alps and A) the altitudinal belt where the parcel is situated, and B) traditional land use. Error bars denote 1 standard error.

Diversity of types of fungal pathogen infection

Per leaf, we recorded 0.26 ± 0.004 of the five types of fungal pathogen infection, and per parcel, we recorded 2.47 ± 0.07. The number of types of fungal pathogen infection per leaf was higher in parcels with higher average leaf damage by fungal pathogen infection (N = 215, R = 0.675, $p < 0.001$, Fig. 1D). Fungal pathogen diversity was highest for graminoids (0.30 ± 0.01 types per leaf), followed by forbs (0.25 ± 0.01) and legumes (0.22 ± 0.01, Fig. 2B).

While the diversity of fungal pathogen types did not differ among altitudes, it was affected by land use. On graminoids in unfertilised sites, we found more types of fungal pathogen infection (0.34 ± 0.02 per leaf) than in fertilised ones (0.23 ± 0.03; functional group by fertilisation interaction, N = 638, $F_{2,199} = 13.1$, $p < 0.001$). Moreover, we recorded more types of fungal pathogen infection on graminoids in abandoned sites (0.45 ± 0.05) than in managed ones (0.27 ± 0.01; functional group by abandonment interaction, N = 638, $F_{2,199} = 11.8$, $p = 0.078$).

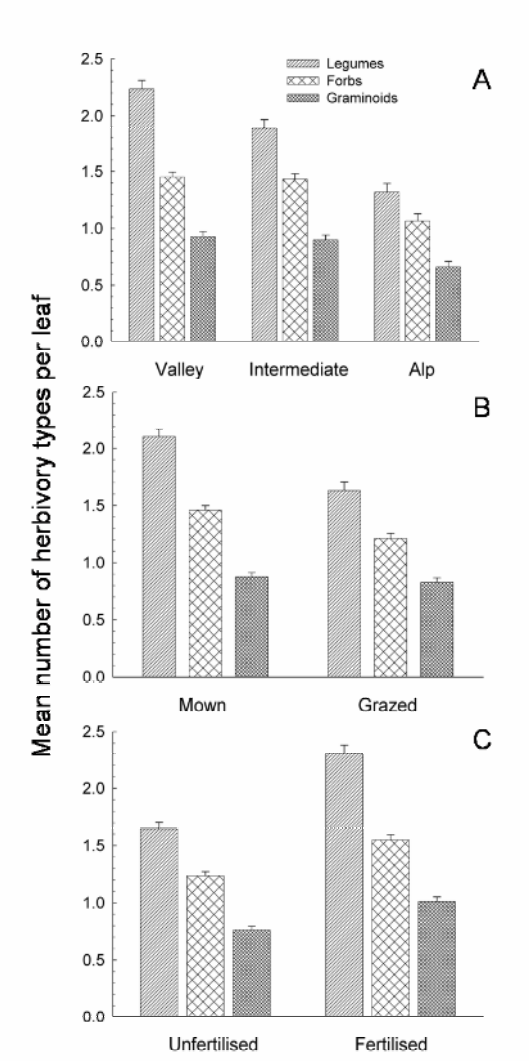

Figure 4 The relationship between the diversity of herbivory per leaf and functional group (legumes, non-legume forbs, graminoids) in 215 grassland parcels in the Swiss Alps and A) the altitudinal belt where the parcel is situated, B) traditional land use, and C) fertilisation. Error bars denote 1 standard error.

Composition of types of damage by herbivory and fungal pathogen infection

Analysis of variance of the principal components of types of damage by herbivory demonstrated significant effects of altitude (PC1, N = 215, $F_{1,101} = 99.0$, $p < 0.001$) and traditional land use (PC1, N = 215, $F_{1,101} = 18.5$, $p < 0.001$) on the composition of these types. This reflects the higher number of eggs on leaves from valleys and intermediate altitudes than from high altitudes (N = 215, $F_{2,212} = 13.8$, $p < 0.001$), and the larger damage by clipping (N = 215, $F_{1,213} = 16.8$, $p < 0.001$), holing (N = 214, $F_{1,101} = 7.8$, $p < 0.01$), and windowing (N = 214, $F_{1,212} = 13.6$, $p < 0.001$) in traditionally mown than in grazed parcels. In contrast to herbivory, the composition of types of fungal pathogen infection was independent of altitude and land use.

Relationships of herbivory and fungal pathogen infection with standing crop and plant species diversity

Standing crop per parcel was positively correlated with the leaf area damaged by herbivory ($N = 209$, $R = 0.184$, $p < 0.001$, Fig. 5A), the number of herbivory types per leaf ($N = 209$, $R = 0.174$, $p < 0.05$, Fig. 5C), the leaf area damaged by fungal pathogen infection ($N = 209$, $R = 0.277$, $p < 0.001$, Fig. 5E), and the number of fungal pathogen infection types per leaf ($N = 209$, $R = 0.433$, $p < 0.001$, Fig. 5G). With increasing number of plant species per parcel the number of herbivory types per leaf decreased ($N = 215$, $R = -0.288$, $p < 0.001$, Fig. 5D).

Discussion

Leaf damage by herbivory and fungal pathogen infection

The infestation of 83% of all sampled leaves demonstrates the ubiquity of plant-herbivore and plant-pathogen interactions in mountain grassland. According to the damaged leaf area, herbivory plays a greater role than fungal pathogens do. However, the low extent of damage by herbivores and fungal pathogens indicates that biomass losses are not substantial. The 2.7% average leaf damage by herbivory is in line with other results showing that leaf damage in 10 herbaceous species in early successional habitats rarely exceeded 3% (Carson & Root, 1999). Such low amounts of leaf damage due to herbivory seem to be quite common (Landsberg & Ohmart, 1989). The observed percentage of leaf damage by fungal pathogens of 1.2% is much higher than the 0.05% damaged leaf area recorded in two tree species (Siemann 2003). However, for pathogens comparable data for grassland are lacking.

The highest leaf damage by herbivory on legumes is in line with the idea that legumes are an important source of nutrient supply for herbivores, and that nitrogen is a limiting nutrient for herbivores (Mattson, 1980). The dominant leaf damage of fungal pathogens on graminoids suggests that factors other than nitrogen affect the preference of fungal pathogens. Possibly, fungal pathogens are dominant on graminoids, because these are more abundant in grassland. Correspondingly, rust species, the most frequent fungal pathogens in our study, are common on pasture grass species (Braverman, 1986).

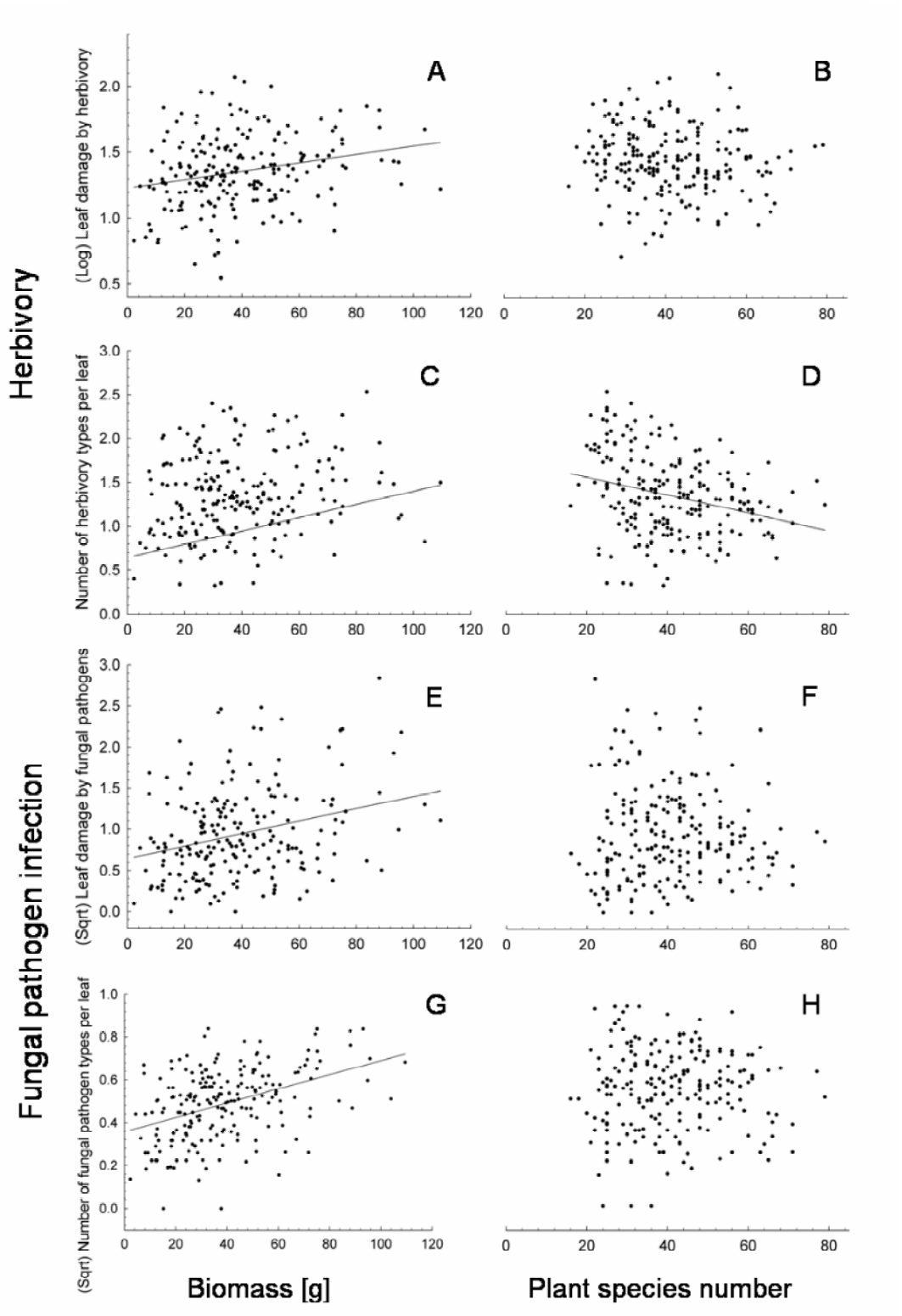

Figure 5 Relationships between leaf damage by herbivory and A) standing crop, B) plant species number, between the diversity of herbivory types per leaf and C) standing crop, D) plant species number, between leaf damage by fungal pathogen infection and E) standing crop, F) plant species number, between the diversity of types of fungal pathogens per leaf and G) standing crop and H) plant species number in 215 grassland parcels in the Swiss Alps. Sqrt denotes square root transformation, log denotes logarithm to the base of ten.

The positive correlation of leaf damage by herbivory and leaf damage by fungal pathogen infection suggests that herbivore damage favours fungal pathogen infection. Insect herbivores can serve as vectors for plant pathogens (Kluth et al., 2002). It was suggested that herbivores affect plants more as vectors of disease than as biomass consumers (Ohnesorge, 1976). However, in our study leaf damage by fungal pathogens was even lower than the one by herbivores. Alternatively, the positive correlation of leaf damage by herbivory and by fungal pathogen infection may reflect that leaf damage by herbivores can facilitate pathogen infection. Whereas the leaf damage by both groups was positively correlated, the diversities of types of herbivory and fungal pathogen infection were independent of each other. This indicates no conflict between the conservation goals of high diversity of herbivores and high diversity of fungal pathogens. Apparently, both groups contribute independently to biodiversity of mountain grassland.

At higher altitudes herbivory damage decreased, while damage by fungal pathogens was not affected by altitude. Lower temperature and shorter season seem to restrict herbivores more than they restrict fungal pathogens. More pronounced herbivory at lower altitudes was also found for grasshoppers (Wettstein & Schmid, 1999) and molluscs (Baur & Raboud, 1988; Scheidel & Bruelheide, 2001). Moreover, in our study especially legume leaves were more damaged by herbivory at lower altitudes, emphasising the importance of this functional group as food supply for herbivores.

The observed higher herbivory damage in traditionally used meadows indicates a preference of herbivores for mown sites, most likely due to frequent disturbances of pastures by trampling by cattle. Accordingly, disturbances in grazed sites were found to negatively influence the presence and establishment of herbivores (Kerley et al., 1993), and intensive grazing was reported to disturb plant-insect interactions (Kruess & Tscharntke, 2002). Thus, in cases where formerly mown grasslands now have to be grazed to reduce labour, the number of cattle and the duration of grazing need to be limited. Microclimatic conditions could also play a role for herbivores, but they were probably not much different between pastures and meadows as this would most likely have affected the level of fungal pathogen infections, which did not differ between pastures and meadows. Finally, as far as damage was caused by flower-visiting insects or their larvae, in the Alps herbivores may prefer mown over grazed grassland, because here mown grassland parcels are usually more flower-rich than grazed ones are (Fischer & Wipf, 2002).

Diversity and composition of types of herbivory and fungal pathogen infection

More types of herbivory on legume leaves, more fungal pathogen types on grass leaves, and more types of herbivory at lower altitudes correspond with the results on leaf damage, and with the positive correlation of the number of types of both herbivory and fungal pathogen infection with leaf damage. Possibly, this indicates a simple mass effect, where a higher number of single plant-parasite interactions is associated both with more severe leaf damage and with a higher likelihood, that a higher number of different herbivore and fungal pathogen groups is involved. Alternatively, it could indicate a direct relationship between higher diversity of interactions and more severe leaf damage. In any case, the damage by single types of herbivory or fungal pathogen infection remained small. Therefore, we conclude that in mountain grassland herbivores and fungal pathogens do not develop the disease-like character described for crop monocultures (Oerke, 1994).

The higher number of types of herbivory at low altitudes is in line with the higher amount of leaf damage there. Most likely it is due to climatic conditions, which may also explain the altitudinal effect on the composition of herbivory types. The above-average number of eggs on leaves from low altitudes reflects the higher reproduction rates of insects under more advantageous climatic conditions (Virtanen & Neuvonen, 1999).

Herbivory was more diverse in mown than in grazed parcels, and the categories with the highest overall frequencies, clipping, holing, and windowing, were also more frequent on mown sites. We conclude that herbivores with these feeding habits prefer mown over grazed grassland. However, the preference of herbivores to specific land use regimes may differ between taxonomic groups. E.g., a more diverse grasshopper community was reported from grazed wetland sites in the pre-Alps, whereas in mown sites the diversity of butterflies was higher (Wettstein & Schmid, 1999). Jeanneret et al. (2003) suggested that there are no general rules relating species diversity to habitat and landscape features, because such relationships strongly depend on the specific organism and study region. Nevertheless, in the Alps mown grassland may be likely to be preferred by herbivores, because meadows are often richer in attractive flowers than pastures (Fischer & Wipf, 2002) and many insect herbivores are flower-visitors.

Increased herbivore diversity may be related to increased productivity of plants via an increase of overall herbivore abundance (Siemann, 1998). Moreover, leaves are generally of higher quality and palatability after nitrogen fertilisation (Davidson & Potter, 1995). Accordingly, we found more different types of herbivory per leaf in fertilised than in unfertilised parcels. In contrast, per parcel the number of types of herbivory did not differ

between fertilised and unfertilised parcels. Most likely in fertilised parcels the higher diversity of herbivory per leaf was counterbalanced by a lower diversity of herbivory between species, because fertilised parcels are less rich in plant species. Accordingly, in experimental grassland the diversity of herbivores increased with the number of weed species (Dyer & Stireman, 2003).

The higher diversity of types of fungal pathogen infection on graminoids in unfertilised grassland, both per leaf and per parcel, most likely is due to an indirect effect of plant species abundance, as in unfertilised sites overall plant species richness is high whereas grass abundance is low. As species specificity restricts the majority of fungal pathogens to one or few closely related host-species (Brandenburger, 1985; Burdon, 1994), we suggest that this specificity might play a greater role for the diversity of fungal pathogen infection than fertilisation does.

Relationship between herbivory, fungal pathogen infection, and standing crop

The positive correlation between standing crop per parcel and leaf damage by, and diversity of types of, herbivory is in line with the preference of herbivores for higher-quality leaves. In unproductive alpine grassland dominated by a single sedge two specialized and very abundant grasshopper species caused heavy biomass losses (Blumer & Diemer, 1996). If, however, plant and herbivore communities are highly diverse, effects of herbivory are found to be dispersed across plant structures and species (Hunter & Price, 1992; Tilman, 1982) and little biomass is consumed. In our grassland parcels, biomass losses remained low even in the fertilised and more productive sites. Possibly, this is due to the still relatively high plant species diversity of these grasslands in the Alps, where the specificity of many herbivores does not allow for large biomass losses. Alternatively, it might reflect positive effects of herbivory on plant growth, as reported for herbivory by insects (McNaughton, 1983) and mammals (Paige, 1992).

Standing crop was positively correlated with the amount of leaf damage by fungal pathogen infection and the diversity of different types per leaf. Possibly, infection is more pronounced due to the higher quality of leaves in more productive sites. Moreover, higher standing crop could promote fungal pathogens because it is associated with higher levels of moisture (Kochy & Wilson, 2004). Furthermore, as higher standing crop was associated with lower plant species diversity and therefore higher abundance per plant species, higher pathogen damage per leaf could also have been due to higher host plant abundance.

At the parcel level, we found no significant relationships between the diversity of different types of herbivory and biomass, and between the diversity of fungal pathogen infection and biomass. This indicates no conflicts between the agricultural goal of high biomass production and the conservation goals of high diversity of fungal pathogens and herbivores.

Relationship between herbivory, fungal pathogen infection, and plant species diversity

At first glance, more types of herbivory per leaf in plant species poor parcels appear to suggest a conservation conflict between plant species diversity and herbivore diversity. However, this relationship disappeared when the diversity of types was considered at the parcel level. Pathogen diversity was also independent of plant species diversity. Therefore, we suggest promoting low-intensity land use where high plant, herbivore, and pathogen diversity contribute to high overall community biodiversity and where biomass losses by herbivores and pathogens are low.

Conclusions

Our comprehensive study across 12 villages from several regions in the Swiss Alps demonstrates the omnipresence of herbivores and fungal pathogens in mountain grassland and indicates that plant-herbivore and plant-pathogen interactions contribute largely to community diversity. At the same time, leaf damage by herbivores and fungal pathogens was very moderate, even in the case of legumes and at lower altitudes where leaf damage was highest. Therefore, conserving the diversity of plant-herbivore and plant-pathogen interactions will not be at the expense of dramatic plant biomass losses.

Our indirect approach of measuring biological interactions allowed us to distinguish between eight categories of leaf damage by herbivores and four frequent taxonomical categories of fungal pathogen infection. This approach enabled us to assess herbivore and fungal pathogen diversity for many field sites at large distances within a short period of time. This worked especially well for plant-herbivore interactions. Because some damage by fungal pathogens could not be classified, the method can be improved for the indirect assessment of plant-pathogen interactions.

Currently, much grassland in the Alps is undergoing land use changes. An increasing proportion of grassland is fertilised and formerly mown grasslands are grazed or abandoned. We found low altitudes and traditional mowing to promote plant-herbivore interactions, while fertilisation negatively affected fungal pathogens. This implies that the current land use changes in the Alps from mowing to grazing or to abandonment decrease the diversity of

plant-herbivore interactions, and fertilisation decreases pathogen diversity. As our results did not reveal conservation conflicts between plants, herbivores, and fungal pathogens, we suggest conserving and maintaining a great diversity of plant-herbivore and plant-pathogen interactions by maintaining diverse low-intensity land use.

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3

Adaptation of the Alpine Meadow Grass *Poa alpina* to altitude and land use in the Swiss Alps

(submitted)

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Abstract

Plants can be adapted to natural and anthropogenic habitat differentiation. However, experimental demonstrations of the adaptiveness of intraspecific diversity are very scarce. Moreover, studies are missing, which simultaneously address several potential drivers of adaptation. We address these issues in the Alpine Meadow Grass *Poa alpina.* The species is widespread in subalpine and alpine habitats in natural sites and on agriculturally used land. Its plants reproduce via seed or via vegetative bulbils. We asked how land use and altitude affect the occurrence of *P. alpina* in the field and whether its performance in the common garden suggests adaptation to conditions at plant origin. First, we screened 216 parcels of land of different land use and altitude (1000-2400 m asl) around 12 municipalities in the Swiss Alps for the presence of *P. alpina*. *Poa alpina* occurred most frequently in fertilized and grazed sites and at higher elevations. Then, we set up a common garden experiment at about 1500 m asl with two plants of each of 615 genotypes originating from 57 grassland parcels and 21 natural sites. One plant per genotype was treated by clipping and one served as control. After two years, by the end of the experiment, nearly 90% of all plants had reproduced. In line with adaptive advantages of vegetative reproduction at higher altitudes, 77 % of reproductive plants from lower altitudes reproduced via seeds, while only 45% of plants from higher altitudes did so. The number of reproductive shoots produced throughout the experiment was highest for genotypes from grazed parcels, whereas clipped and final vegetative biomasses were highest for plants from mown parcels. Accordingly, in line with adaptive advantages of reproduction in grazed sites, which offer more gaps for establishment, allocation to reproductive biomass was higher in plants from grazed parcels than from mown parcels. The intraspecific differences between plants originating from parcels of different altitude and land use indicate that phenotypic differentiation of *P. alpina* in the field has a genetic component, which is shaped by adaptation to both natural and anthropogenic influences.

Key words: common garden experiment; genetic diversity; plasticity; reproduction; grassland;

Introduction

While it is theoretically clear that plants can be adapted to natural and anthropogenic habitat differentiation, experimental demonstrations of the adaptiveness of intraspecific diversity are very scarce (Callaway et al. 2003, Pigliucci 2003, Santamaria et al. 2003). Moreover, studies are missing, which simultaneously address several potential drivers of adaptation. Effects of natural and anthropogenic habitat differentiation on genetic within-species diversity should be especially important and apparent for species that are distributed across wide altitudinal and land use ranges. Such a species is the Alpine Meadow Grass *Poa alpina* L. (Poaceae) which is among the most frequent grasses in the European Alps (Conert 1998). It is widespread in mountain meadows and pastures and at natural sites such as rock fans, snow beds, and riverbeds. Moreover, it is one of the most important fodder grasses of the European Alps (Conert 1998).

The heterogeneous landscape of the Alps is characterized by pronounced natural, especially altitudinal, gradients (Theurillat et al. 2003). For thousands of years, this landscape and especially its grassland has also been shaped by different cultural and agricultural traditions of human land use (Ellenberg 1996, Bätzing 2003). This natural and anthropogenic heterogeneity is reflected in high landscape and community diversity within and between sites, and some studies have been directed towards explaining effects of this heterogeneity for vegetation composition (Guido and Gianelle 2001, Grabherr 2003). In the Swiss Alps, old cultural traditions, namely the Romanic, Germanic, and Walser ones, as well as recent land use changes affect vegetation diversity and composition (Chapter 1). Such land use changes are particularly important, as in the Alps anthropogenic land use is undergoing large socio-economically motivated change (Bätzing 1991, 1993) involving both abandonment of land and intensification of agriculture (Olsson et al. 2000, Fischer and Wipf 2002). In contrast to between-species diversity and despite the presumably strong selection factors that natural and anthropogenic drivers constitute, their effects on within-species diversity received little attention.

Selection by different types of land use over hundreds of years is likely to have caused adaptive variation of growth and reproduction of *P. alpina*. Compared with natural and grazed sites, vegetative growth is likely to be favored under high levels of competition in mown grassland with their dense homogeneous swards (Abrahamson 1980). Alternatively, in very dense vegetation, such as the one in abandoned parcels, selection may favor reproduction as means of producing propagules for escape (Abrahamson 1980, Fischer and van Kleunen 2001).

Higher allocation to reproduction may have evolved in grazed grassland, where gaps in the vegetation favour recruitment more than homogeneous mown grassland does. *Poa alpina* occurs in two reproductive forms. Seminiferous plants produce seeds, while pseudoviviparous plants produce bulbils vegetatively, which develop into little plantlets on the parental plant (Pierce et al. 2000). Vegetative reproduction has been suggested to be of adaptive advantage at higher altitudes, where climatic conditions do not favour seedling establishment (Körner 2003).

While it is likely that long-term selection has led to genetic differentiation, phenotypic variation can additionally be shaped by plastic responses to land use. To some degree the mode of reproduction appears to be phenotypically plastic, as was demonstrated in experiments where plants turned pseudoviviparous at short day conditions and cold temperatures (Schwarzenbach 1953, Heide 1989). Moreover, plasticity can be adaptive in heterogeneous environments, where we expect plants to be more plastic, e.g. in response to disturbance brought about by land use, than plants from more uniform conditions (Fischer and van Kleunen 2001). Consequently, as grazed grasslands are more heterogeneous than mown ones, we expect higher such plasticity in plants from grazed sites, particularly for reproductive characters.

A powerful tool to partition phenotypic variation into genetic and environmental components is provided by experimentation in the common garden, where plant growth, plant response to simulated land use, and interactions between plant origin and simulated land use can be studied under uniform conditions (Silvertown and Charlesworth 2001).

We studied the effects of altitude and land use on the occurrence of *P. alpina* in more than 200 grassland parcels and on its growth and reproduction in a common garden experiment. To also study effects of origin on plasticity, we included a clipping treatment simulating land use. For the experiment, we used clonally derived plants from 615 genotypes from 21 natural sites and 57 grassland parcels. These sites represent different altitudes and land use around 12 municipalities along an east-west gradient of about 170 km. To account for potential regional and cultural differences, we selected four villages of each of the three main cultural traditions in the Swiss Alps, Romanic, Germanic, and Walser. We asked the following specific questions: (i) In the field, how does the occurrence of *P. alpina* depend on altitude and land use? In the experiment, (ii) do plants from higher altitudes rather reproduce via bulbils than via seeds, (iii) do plants from mown parcels allocate more to vegetative growth, (iv) do plants from grazed parcels allocate more to reproduction, and (v) are plants

from grazed grassland more phenotypically plastic in response to experimental clipping than plants from more homogeneous abandoned or mown grassland are?

Methods

Species

The Alpine Meadow Grass *Poa alpina* L. (Poaceae) is widespread in upland regions in the Northern hemisphere, and locally at low altitudes in the North, in exposed grasslands and snow beds (Conert 1998). It occurs mainly on rich soils or alluvial plains and indicates high levels of nutrients and moisture. As a pioneer species, it also colonizes rock fans, other stony and rocky places of calcareous and siliceous rocks, and exposed slopes and ridges. In the Swiss Alps, it occurs in natural sites up to 4200 m asl and in agriculturally used land between 550 and 2500 m asl (Conert 1998). Because of its high fat and protein contents *P. alpina* is one of the most important fodder grasses in mountain meadows and pastures (Conert 1998).

The species is perennial and occurs in seminiferous and pseudoviviparous forms. The latter ones reproduce by proliferation of the spikelet axis and production of bulbils instead of seeds. Some seminiferous plants produce seeds sexually, others via apomixis (Müntzing 1933).

Occurrence of the species

We studied the occurrence of *P. alpina* in agricultural grassland around 12 villages in the Swiss Alps, four of each of the three cultural traditions (named after the original settling tribes) Romanic, Germanic, and Walser. Each village is part of a separate alpine valley, and the 12 villages are located along an east-west gradient of about 170 km. To exclude regions with extreme landscape changes we selected villages whose agriculture has only changed modestly during the last 50 years, that are not very touristy, and that have not grown to more than 1500 inhabitants. At three altitudinal levels per village (valley at about 1000 m asl, intermediate altitudes about 1500 m asl, and alp at about 2000 m asl), we had selected parcels of land representing different land use combinations (Chapter 1). These combinations comprised parcels that were fertilized or unfertilized, had traditionally been mown or grazed, and were currently mown, grazed, or abandoned. In close collaboration with local farmers, we had found 147 out of 432 possible combinations of village, altitude, and traditional and current land use. At some altitudes in some villages were no parcels whose current land use differed from the traditional one. In these cases, we selected two parcels of the concerned type

of traditional land use to increase sample size. In total, we selected 216 grassland parcels, between 12 and 24 per village, and screened them for the presence of *P. alpina*.

Plant material

For our common garden experiment, we used plants of *P. alpina* from 65 grassland parcels where land use had not changed. In each parcel, we sampled eight plants, plus two extra plants for potential replacement, at interdistances of at least 5 m. In addition, we sampled the same number of plants of *P. alpina* from 24 natural sites, two per village. We considered sites as natural, when they were located within or adjacent to natural plant communities such as the *Caricetum curvulae* or the *Caricetum firmae* (Ellenberg 1996), and when they were at least 200 meters separated from agriculturally used grassland.

Plant propagation

Because plant samples consisted of several tillers, we isolated single genotypes from each plant sample. To this end, first we separated each field-collected plant into four single tillers, which we planted into the corners of 7 cm x 7 cm pots. As substrate, we used a 1:2 mix of sand and standard potting turf soil. We grew the plants in a greenhouse. After two months, in October 2002, we repotted one randomly selected plant grown out of the four single tillers, and discarded of the others. Then, after another 5 months, we divided the plants to receive two replicates per genotype and planted them each into new pots. After leaving the plants for 2 weeks in the greenhouse, we transferred them to the university garden (450 m asl) for outdoor acclimatization before planting them to the experimental site in the Alps. Four weeks later, in May 2003, immediately before transferring the plants to the experimental site, we measured initial size as number of tillers, diameter of the thickest tiller, and, if applicable, we recorded the mode of reproduction for each plant. At the same time, we clipped the plants to a standard height of 12 cm above ground to further reduce potential carry-over effects.

Experimental design

We used the plants to set up a common garden experiment in a formerly agriculturally used field at about 1500 m asl at Davos in the Swiss Alps in May 2003. In total, we planted 1380 plants (two plants of each of 690 genotypes) in a grid pattern in eight blocks, each consisting of four rows with up to 44 plants. The space available for each plant was 20 cm x 20 cm. For convenient application of a clipping treatment simulating land use to one of the plants per genotype, we planted block-wise in four blocks per treatment. The other plant per genotype in

the remaining four blocks (alternating with the clipping-treatment blocks) served as control. Within blocks, plants were randomly assigned to planting positions in the grid. Monthly weeding prevented other plants – including *P. alpina* plants growing from seed or bulbils – from establishing within the experiment.

Measurements

In the clipping-treatment blocks, we harvested vegetative biomass (later on called clipped biomass) by clipping all leaves 5 cm above ground, but leaving out emerging reproductive shoots. In 2003, we clipped twice, in July and in October. In 2004, we clipped and harvested the vegetative biomass of the plants in the clipping-treatment blocks once at the end of June. One month later, we finished the experiment by harvesting the aboveground vegetative biomass of all plants, including the control plants, at 3 cm above ground (later on called final vegetative biomass). After the harvest, we dried the biomass at 80 °C and weighed it.

We harvested reproductive biomass of each plant for 2 years. In 2003, we harvested monthly from July to October. Of each reproducing plant, we cut off the reproductive shoots 5 cm above ground, counted them, and collected them in paper bags. Moreover, we noted the reproductive mode as seminiferous or pseudoviviparous. The reproductive shoots were only taken when at least a few anthers were visible (then noted as seminiferous), or when bulbils were well developed and about to dehisce (then noted as pseudoviviparous). Less developed shoots were left for the next harvest. For each plant, we harvested all reproductive biomass into the same paper bag to cumulatively receive the total amount of reproductive biomass. After drying the reproductive biomass at 80 °C, we weighed it and summed up the number of shoots. In 2004, we harvested reproductive biomass at the end of June and at the time of final harvest.

Statistical analysis

We analyzed the effects of land use and altitude on the occurrence of *P. alpina* in the field by logistic regression based on binomial distribution. The full hierarchical mixed model included the factors culture (referred to as cultural tradition), village (nested in culture), altitude, fertilization, abandonment, current land use, and all twofold interactions.

For the analyses of the experiment, 75 plants were not taken into account due to wrong sampling information. Moreover, independent of the experimental factors, another 75 plants died during the experiment and were not taken into account. Finally, of the originally 1380

plants, we included 1230 plants in the analyses, 905 from 57 grassland parcels and 325 from 21 natural sites.

For the clipping-treatment plants, we analyzed cumulative clipped vegetative biomass, cumulative clipped reproductive biomass, and cumulative number of clipped reproductive shoots. These cumulative numbers did not include the final harvest. The full hierarchical mixed ANOVA model included the factors culture, village (nested in culture), altitude, natural (referred to as natural sites), fertilization, abandonment, current land use, including all interactions and parcel (Table 1). From this full model, we selected reduced best models for each variable using Akaike's Information Criterion (AIC) -values (Burnham and Anderson 2002). This model selection procedure revealed as best model the one comprising the main seven factors culture, village, altitude, natural, fertilization, current land use, parcel, without any interactions (Table 1).

For all plants, we analyzed cumulative reproductive biomass and cumulative number of reproductive shoots, final vegetative biomass, total vegetative biomass (i.e. sum of final harvest and clipped biomass), and percentages of reproductive and vegetative biomass (relative to the sum of vegetative and reproductive biomass) with the same model as above, but adding the factors genotype and treatment. Again, we selected reduced best models for each variable starting with the full hierarchical model including all interactions. Finally, the best model included the main seven factors culture, village, altitude, natural, fertilization, current land use, parcel, and their two-way interactions with treatment (Table 1). Final vegetative biomass and total vegetative biomass were log-transformed, and reproductive biomass and number of reproductive shoots were square root transformed prior to analysis of variance (ANOVA) to meet ANOVA assumptions.

Table 1 Selected best ANOVA models for the analysis of common-garden variation in A) clipped vegetative biomass, B) reproductive biomass, number of reproductive shoots, final vegetative biomass, percentage of reproductive biomass, and total biomass. The analyses involved 1230 *Poa alpina* plants from 57 grassland parcels of different altitude and land use and 21 natural sites in 12 villages of three different cultural traditions in the Swiss Alp, except for the analysis of clipped biomass, which involved 612 such plants. Note: MS = mean square, F = variance ratio.

A	Source of variation	MS	F
	Culture	ms_{cult}	ms_{cult} / ms_{vill}
	Village	ms_{vill}	ms_{vill} / ms_{parcel}
	Altitude	ms_{alt}	ms_{alt} / ms_{parcel}
	Natural	ms_{nat}	ms_{nat} / ms_{parcel}
	Fertilization	ms_{fert}	ms_{fert} / ms_{parcel}
	Current land use	ms_{curr}	ms_{curr} / ms_{parcel}
	Parcel	ms_{parcel}	$ms_{parcel} / ms_{residual}$
	Residual = Plant	$ms_{residual}$	

B	Source of variation	MS	F
	Culture	ms_{cult}	ms_{cult} / ms_{vill}
	Village	ms_{vill}	ms_{vill} / ms_{parcel}
	Altitude	ms_{alt}	ms_{alt} / ms_{parcel}
	Natural	ms_{nat}	ms_{nat} / ms_{parcel}
	Fertilization	ms_{fert}	ms_{fert} / ms_{parcel}
	Current land use	ms_{curr}	ms_{curr} / ms_{parcel}
	Parcel	ms_{parcel}	$ms_{parcel} / ms_{genotype}$
	Genotype	ms_{geno}	$ms_{geno} / ms_{residual}$
	Clipping	ms_{clip}	$ms_{clip} / ms_{residual}$
	Clipping x Culture	$ms_{clip \times cult}$	$ms_{clip \times cult} / ms_{clip \times vill}$
	Clipping x Village	$ms_{clip \times vill}$	$ms_{clip \times vill} / ms_{clip \times parcel}$
	Clipping x Altitude	$ms_{clip \times alt}$	$ms_{clip \times alt} / ms_{clip \times parcel}$
	Clipping x Natural	$ms_{clip \times nat}$	$ms_{clip \times nat} / ms_{clip \times parcel}$
	Clipping x Fertilization	$ms_{clip \times fert}$	$ms_{clip \times fert} / ms_{clip \times parcel}$
	Clipping x Current land use	$ms_{clip \times curr}$	$ms_{clip \times curr} / ms_{clip \times parcel}$
	Clipping x Parcel	$ms_{clip \times parcel}$	$ms_{clip \times parcel} / ms_{residual}$
	Residual = Clipping x Genotype	$ms_{residual}$	

To test variation in whether plants reproduced at all, we used logistic regressions based on binomial distribution. To analyze which factors affect the mode of reproduction we summed the number of reproducing plants per treatment and parcel and calculated the percentage of seminiferously reproducing plants, arcsin transformed it and fitted an ANOVA model including effects of culture, village, altitude, natural, fertilization, current land use, parcel, treatment, and all interactions of the treatment.

We analyzed all data separately for each year (by summing both harvests of clipped biomass and all harvests of reproductive biomass respectively number of reproductive shoots per year) and for both years combined. In all analyses, we treated village, parcel, and genotype as random factors. Including initial tiller diameter, number of tiller, and row and position in the experiment as covariates did not change levels of significance. Therefore, we report the results of models without covariates. We calculated all logistic regressions and ANOVAs with the program GENSTAT (Version 6.1, Lawes Agricultural Trust, 2002).

Results

Occurrence of Poa alpina in the field

Poa alpina occurred in parcels representing 85 out of the 147 combinations of village, altitude, and land use. It occurred more frequently in parcels at higher altitudes than at lower ones ($N = 147$, $F_{2,130} = 12.57$, $p < 0.001$, Fig. 1A). Moreover, it occurred more frequently in fertilized parcels than in unfertilized ones ($N = 147$, $F_{1,130} = 12.88$, $p < 0.001$, Fig. 1B). Finally, *P. alpina* occurred most frequently in grazed parcels, followed by mown and abandoned ones ($N = 147$, $F_{2,130} = 25.87$, $p < 0.001$, Fig. 1B). In summary, higher altitude, fertilization, and grazing favored the occurrence of *P. alpina*.

Vegetative growth in the common garden

In 2003 and 2004 and for both years and combined, clipped vegetative biomass was highest for plants from mown sites (ANOVA of combined clipped biomass: $N = 612$, $F_{2, 61} = 5.31$, $p < 0.01$) and lowest for plants from grazed and abandoned sites. Whereas in 2003 and for both years combined, clipped plants from natural sites produced the second highest vegetative biomass, they produced the least in 2004. While the above-mentioned differences between plants from mown and grazed sites were significant, those between plants from natural, used, and abandoned ones were not. Altitude of origin did not affect vegetative growth in the common garden.

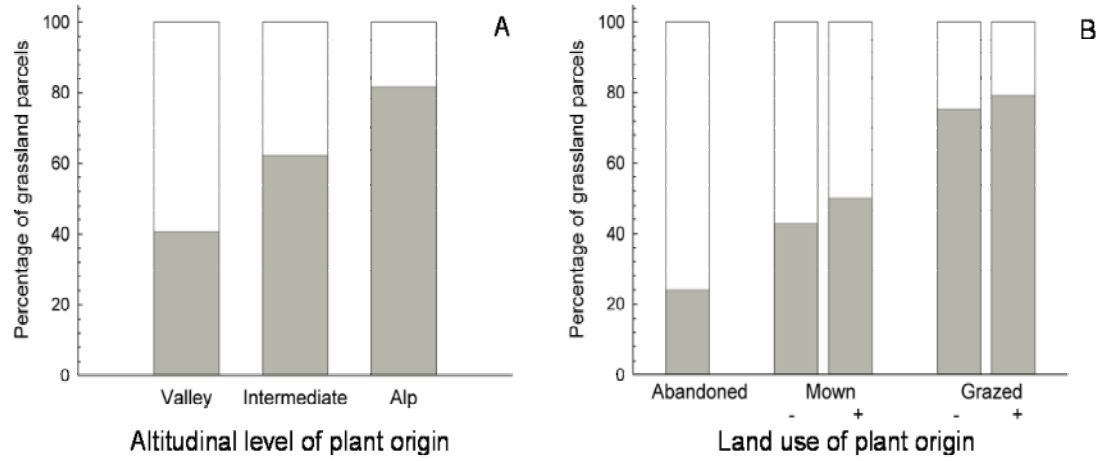

Figure 1 Proportion of studied grassland parcels in the Swiss Alps in which *Poa alpina* occurred according to A) three altitudinal levels where the parcels were situated, and B) land use of the parcels. Fertilized parcels indicated by +, unfertilized by –. Shaded = *P. alpina* present, open = *P. alpina* absent.

At final harvest, control plants yielded more final vegetative biomass than clipped plants (N = 1230, $F_{1,536} = 180.26$, $p < 0.001$, Fig. 2), although the percentage of vegetative biomass of total biomass was higher in clipped plants than in control plants (N = 1210, $F_{1, 516} = 130.18$, $p < 0.001$). Final vegetative biomass was higher in plants from mown grasslands (N = 1230, $F_{2,61} = 5.74$, $p < 0.01$, Fig. 2) than in plants from grazed, natural, and abandoned ones. Moreover, at final harvest, plants originating from fertilized parcels yielded more final vegetative biomass than those from unfertilized ones did (N = 1230, $F_{1,61} = 4.26$, $p < 0.05$). In summary, mowing and fertilization of the parcel of origin enhanced the common garden yield of final vegetative biomass.

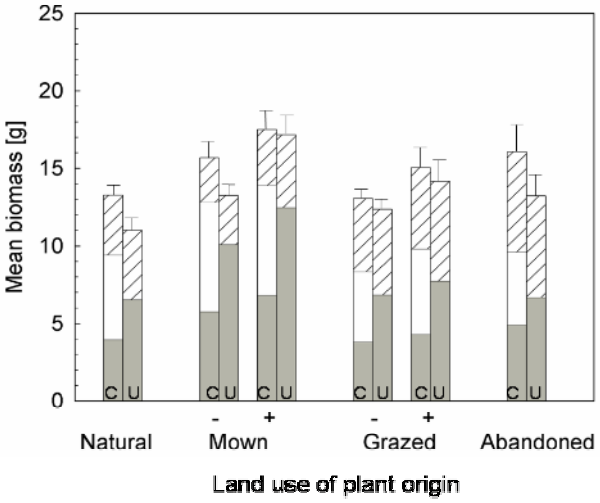

Figure 2 Overview of biomass produced in the common garden experiment with 1230 plants of *Poa alpina* originating from grassland parcels of different land use. Final vegetative biomass is indicated by shaded portions of bars, clipped biomass by open portions of bars, and reproductive biomass by hatched portions of bars. C denotes clipped plants, and U unclipped plants. Error bars denote 1 S.E. of total biomass.

Table 2 Vegetative and reproductive biomass in the common garden, and number of reproductive shoots and their means ± SE and number N of *Poa alpina* plants originating from grassland parcels of different land use in the Swiss Alps, combined over both years of the experiment and for the years 2003 and 2004 separately. Total biomass is the sum of vegetative and reproductive biomass. Numbers in brackets denote number of parcels of plant origin, and fertilized +, unfertilized -. Note: As not every plant reproduced every year, for the reproductive characters numbers and means of 2003 and 2004 do not add up to both years combined.

	Year	Natural (21)		Mown - (8)		Mown + (6)		Grazed - (29)		Grazed + (11)		Abandoned (4)	
		Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
Total biomass [g]		12.87 ± 0.52	325	14.92 ± 0.68	128	17.74 ± 0.9	93	12.97 ± 0.42	448	15.09 ± 0.94	172	15.01 ± 1.15	64
Final vegetative biomass [g]		5.25 ± 0.32	325	7.93 ± 0.344	128	9.68 ± 0.61	93	5.32 ± 0.24	448	6.02 ± 0.45	172	5.79 ± 0.54	64
Clipped vegetative biomass [g]	combined	5.45 ± 0.28	162	7.05 ± 0.47	64	7.08 ± 0.40	46	4.52 ± 0.22	224	5.51 ± 0.46	85	4.69 ± 0.59	32
	2004	0.92 ± 0.06	162	1.78 ± 0.18	64	1.78 ± 0.14	46	1.11 ± 0.06	224	1.36 ± 0.14	85	1.15 ± 0.10	32
	2003	4.53 ± 0.25	162	5.27 ± 0.35	64	5.30 ± 0.34	46	3.41 ± 0.19	224	4.15 ± 0.36	85	3.54 ± 0.54	32
Reproductive biomass [g]	combined	6.50 ± 0.60	274	3.01 ± 0.31	108	4.19 ± 0.57	85	5.13 ± 0.23	419	5.80 ± 0.47	151	4.17 ± 0.30	60
	2004	4.07 ± 0.42	241	2.66 ± 0.25	106	3.18 ± 0.43	85	3.38 ± 0.17	387	3.91 ± 0.36	141	2.77 ± 0.21	60
	2003	3.65 ± 0.41	174	2.43 ± 0.54	18	3.41 ± 0.63	25	2.95 ± 0.16	286	3.45 ± 0.30	94	2.73 ± 0.25	40
Number of reproductive shoots	combined	55.7 ± 5.1	274	26.3 ± 2.9	108	24.9 ± 2.6	85	47.4 ± 2.1	419	48.8 ± 3.7	151	40.7 ± 2.8	60
	2004	41.2 ± 4.1	241	24.9 ± 2.6	106	22.0 ± 2.5	85	36.9 ± 1.8	387	37.5 ± 3.1	141	33.4 ± 2.1	60
	2003	21.7 ± 2.6	174	11.5 ± 2.9	18	9.7 ± 1.5	25	19.5 ± 1.2	286	22.2 ± 1.7	94	17.8 ± 1.9	40

Reproduction in the common garden

By the end of the experiment, 89.3% of the 1230 plants had reproduced. In 2003, 52.2% of all plants reproduced, and in 2004 82.9% reproduced. Overall, unclipped plants produced more reproductive biomass (5.12 g ± 0.23) than clipped ones did (4.43 g ± 0.19; N = 1097, $F_{1,61}$ = 14.43, $p < 0.001$).

In 2003, plants from grazed sites were more likely to reproduce than plants from mown and abandoned ones (N = 1230, $F_{2,61}$ = 5.80, $p < 0.01$). In 2004 and for the two years combined, land use of the parcel of origin did not affect the likelihood of reproduction.

The number of reproductive shoots was closely positively correlated with reproductive biomass (N = 1097, R = 0.862, $p < 0.001$). Both the number of reproductive shoots (for both years combined: N = 1097, $F_{1,61}$ = 12.97, $p < 0.001$ Fig. 3A) and reproductive biomass decreased with increasing altitude of origin (for both years combined: N = 1097, $F_{1,61}$ = 13.20, $p < 0.001$, Fig. 3B). Moreover, the percentage of reproductive biomass decreased with increasing altitude (N = 1097, $F_{1,61}$ = 25.96, $p < 0.001$).

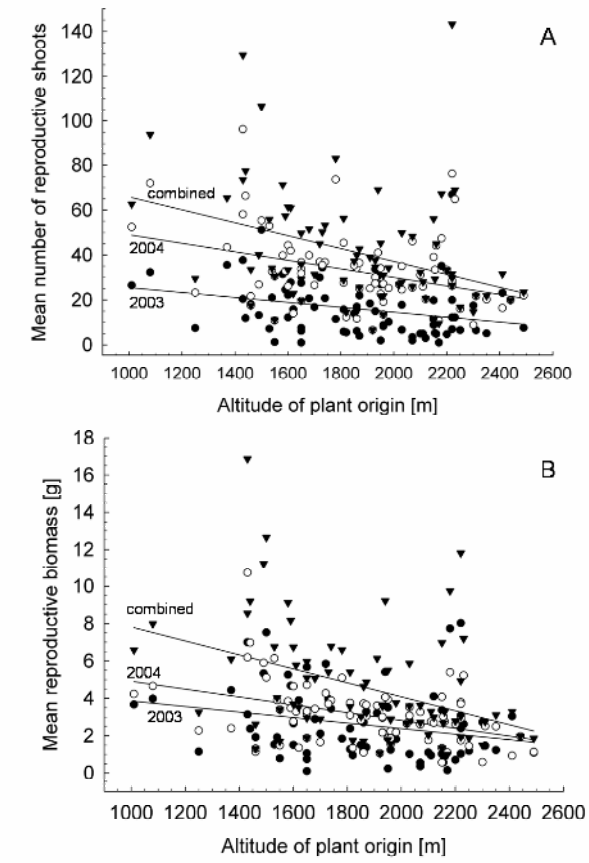

Figure 3 A) Mean number of reproductive shoots, and B) mean reproductive biomass of *Poa alpina* plants originating from grassland parcels at different altitude grown in the common garden. Filled circles denote plants reproducing in 2003, open circles in 2004, and triangles the combination over both years (i.e. plants reproducing in both or either year).

More reproductive shoots were produced by plants from grazed sites than by plants from mown and abandoned ones (N = 1097, $F_{2,61}$ = 4.81, p < 0.05). Land use did not have any effects on the amount of reproductive biomass, whereas it affected the percentage of reproductive biomass. In both treatments, compared with plants from natural sites, allocation towards reproduction was significantly increased in plants from grazed sites and decreased in plants from mown ones (N = 1210, $F_{2,61}$ = 7.53, p = 0.001, Fig. 4).

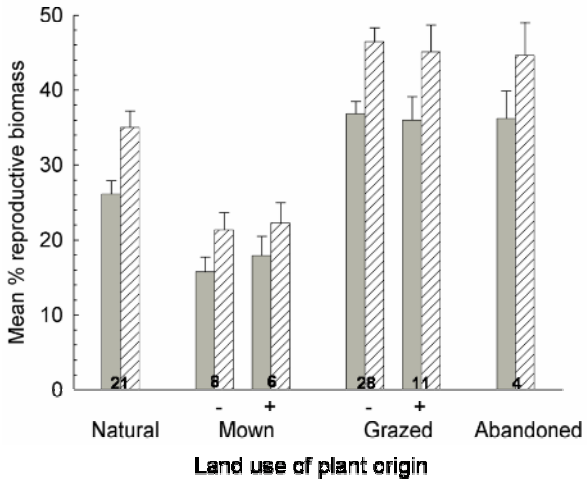

Figure 4 Mean percentage of reproductive biomass relative to total biomass of clipping treatment plants (shaded) and control (hatched) plants of *Poa alpina* originating from grassland parcels of different land use grown in the common garden. Numbers within bars denote number of parcels of origin of the types of land use. Fertilized parcels indicated by +, unfertilized ones by –. Error bars denote 1 S.E.

More plants per parcel originating from low altitudes reproduced via seed, whereas pseudoviviparous reproduction dominated in plants from high altitudes (N = 156, $F_{1,61}$ = 8.37, p < 0.01, Fig. 5). Land use of the parcel of plant origin did not affect the mode of reproduction. We recorded only two plants that changed their reproductive mode from one year to another (one from seminiferous to pseudoviviparous, one vice versa). Different reproductive modes between the two plants of the same genotype occurred six times.

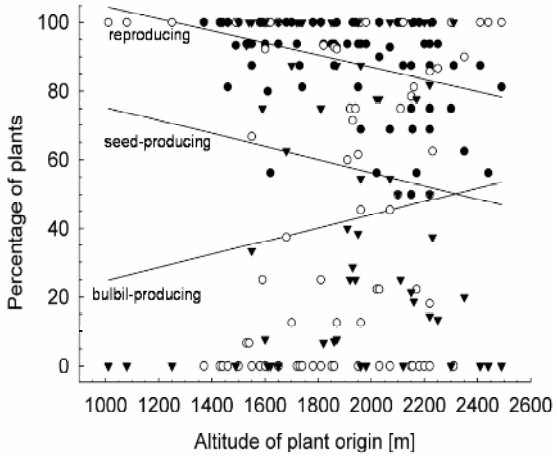

Figure 5 Total proportion of reproducing *Poa alpina* plants and proportion of plants per reproductive mode (filled circles = reproducing, open circles = reproducing by bulbils, triangles = reproducing by seeds) in the common garden in relationship to the altitude of the parcel of plant origin.

Discussion

Habitats of Poa alpina

The higher likelihood of occurrence of *P. alpina* in grazed sites suggests a preference for more disturbed sites where vegetation gaps facilitate establishment of seedlings (Grubb 1977) or plantlets derived from bulbils. This corresponds to the results that plants in the common garden originating from grazed parcels produced more reproductive shoots and higher reproductive biomass than plants from other parcels (see below).

The higher likelihood of occurrence of *P. alpina* at higher altitudes corresponds well with its described distribution in subalpine habitats (Conert 1998). Our finding of higher likelihood of occurrence of *P. alpina* in fertilized grassland also confirms its preference for nutrient-rich habitats, which is also indicated by a high nutrient indicator value of the species (Ellenberg et al. 1992).

Evidence for adaptation of vegetative growth

In our experiment, plants from mown grassland produced more vegetative biomass than plants from grazed sites did, suggesting divergent selection between mown and grazed sites. We suggest that, as hypothesized, high vegetative growth rates are competitively favorable in mown grassland where biomass is removed regularly. On the other hand, plants with high vegetative growth rates would be more conspicuous and therefore run higher risks of being consumed in grazed sites, which reduces the potential competitive advantage of fast growth. These differences in common-garden growth of plants from mown and grazed parcels suggest adaptation of *P. alpina* to land use. Similarly, intraspecific variation of the grass *Sesleria albicans* in a common garden was related to habitat quality and land use of its anthropogenic habitats of origin (Reisch and Poschlod 2003).

The absence of interacting effects on vegetative growth of clipping treatment and land use at the site of origin indicates that *P. alpina* did not adapt to different land use regimes by differences in plastic response to biomass removal.

Some previous studies of grassland plants report overcompensation of biomass removal by increased growth (Lennartsson et al. 1998, van Kleunen et al. 2004). Correspondingly, in our experiment previously clipped plants yielded a higher total biomass than previously unclipped plants indicating that compensatory growth also plays a role in *P. alpina.*

Evidence for adaptation of reproduction

Lower allocation to reproduction of plants from higher altitudes is in line with the hypothesis, that harsher conditions for seedling and bulbil establishment at higher altitudes cause an adaptive advantage of allocation to vegetative growth. In general, higher rates of plant establishment are expected in pastures due to their spatial heterogeneity and higher density of regeneration gaps (Grubb 1977). The higher likelihood of plants from grazed parcels than from mown parcels to reproduce and their higher biomass allocation to reproduction are likely to constitute adaptations to take advantage of the increased availability of open sites for establishment created by grazers and may explain part of the association of *P. alpina* with grazed habitats.

The absence of interacting effects on reproduction of clipping treatment and land use at the site of origin indicate that adaptation of reproduction of *P. alpina* to land use does not involve phenotypic plasticity.

Higher rates of reproduction of plants from grazed parcels do not only have demographic consequences, but are also likely to increase genetic diversity (Machon et al. 2001) due to increased seedling recruitment and higher numbers of dispersed propagules. In the case of *P. alpina*, genetic diversity measured with microsatellite markers (Chapter 5) and measured as broad-sense heritabilities of several quantitative traits (Chapter 6) was indeed higher for grazed parcels than it was for mown parcels. An alternative explanation for higher genetic diversity among genotypes from grazed parcels could be more monotonic selection in more homogeneous mown parcels than in more heterogeneous grazed parcels. Accordingly, an intensive cutting regime reduced genetic variability for *Festuca pratensis* (Kölliker et al. 1998).

Seed production and establishment of seedlings can be a rather risky mode of propagation at high altitudes (Bliss 1972, Billings 1974), which may be even more risky than establishing from bulbils. Therefore, the higher percentage of pseudoviviparously reproducing plants from higher altitudes may indicate an adaptation to climatic limitations endangering seed recruitment. The shorter growth periods at higher altitudes are more suitable for pseudoviviparously reproduced offspring, as bulbil-derived plantlets are supported by the maternal plant longer than seeds are and therefore can grow into mature plants much faster than seedlings can (Harmer and Lee 1978).

Apart from being adapted to altitudinal conditions, the mode of reproduction could also respond plastically to changing environments. Previous studies with *P. alpina* reported plastic responses by switching from pseudoviviparous to seminiferous reproduction after

transplanting pseudoviviparous plants from cold to moderate temperatures (Schwarzenbach 1953, Bachmann 1980). However, in these studies, the majority of plants did not switch their mode of reproduction at all. Such switches seem to be the exception, as in our study, only two plants (0.16%) switched their reproductive mode between 2003 and 2004 respectively only six genotypes (0.98%) between the clipped and control replicate plant. Therefore, as far as these test environments are representative for natural variation, the mode of reproduction appears largely determined genetically in *P. alpina.*

In abandoned parcels with their high levels of standing crop, high reproductive effort is less likely to reflect an adaptation taking advantage of comparatively low competition. Therefore, the highest number of reproductive shoots in plants from abandoned sites might reflect an adaptation according to the alternative strategy, where increased production of propagules under high competition enables escape (Abrahamson 1980, Fischer and van Kleunen 2001, van Kleunen et al. 2001).

Some studies on manipulated losses of reproductive organs reported at least compensatory responses in reproductive traits (Gedge and Maun 1994, Gomez and Fuentes 2001). In our study, the lower reproductive biomass in clipped plants indicates that the removal of vegetative biomass also reduced reproduction. Thus, there is no evidence for compensatory reproduction following biomass removal in *P. alpina*.

Conclusions

Our study demonstrates that in the heterogeneous landscape of the Alps, *P. alpina* is adapted not only to the natural altitudinal gradient, but also to anthropogenic land use variation. This implies that the ongoing socio-economically motivated change of agricultural land use in grassland of the Swiss Alps will not only change vegetation composition and diversity, but also adaptive intraspecific diversity of the widespread grass *P. alpina,* and most likely also of other species.

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4

Isolation and characterization of microsatellite DNA markers in the grass *Poa alpina* L.

(Molecular Ecology Notes, doi: 10.1111/j.1471-8286.2005.01019.x)

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Abstract

The important fodder grass *Poa alpina* L. occurs at several ploidy levels with common aneuploidy. We isolated and characterized five polymorphic microsatellite markers for the study of molecular genetic variation of this species. As first examples of the value of the developed markers for population genetic analyses we show that plants with more chromosomes have more microsatellite bands and that isolation by distance plays a small role in shaping microsatellite diversity of *P. alpina* in the Swiss Alps**.**

Keywords: Microsatellite, *Poa alpina*, population genetics

The Alpine meadow-grass, *Poa alpina* L., is a common species of subalpine and alpine grasslands. The species constitutes an aggregate of phenotypically diverse polyploid ecotypes with common aneuploidy (Müntzing 1980). Chromosome numbers range from 2n = 22 to 46 in Switzerland (Duckert-Henriod & Favarger 1987) to more than 60 in Scotland (Müntzing 1980). Some plants of *P. alpina* reproduce via seeds, while others reproduce vegetatively forming bulbils in the panicles instead of seeds. It is not completely clear to which degree the mode of reproduction is genetically determined or phenotypically plastic.

We extracted DNA from leaf samples of eight plants originating from a natural population in a pasture at 1950 m asl in Pany, Switzerland. Total genomic DNA was extracted using a phenol-chloroform extraction procedure (Rogers & Bendich 1994) modified by Steinger *et al.* (1996), except that we incubated the samples mixed with cetyltrimethyl ammonium bromide (CTAB) buffer and mercaptoethanol at 65°C.

In the lab of ecogenics GmbH (Zurich, Switzerland), we produced an enriched DNA library from size-selected genomic DNA ligated into TSPAD-linker (Tenzer *et al.* 1999) and enriched by magnetic beads selection with biotin-labelled (CA)13 and (GA)13 oligonucleotide repeats (Gautschi *et al.* 2000a; Gautschi *et al.* 2000b). Out of the 384 screened recombinant colonies, 98 gave a positive signal after hybridization. We sequenced plasmids from 80 positive clones as described in Gautschi *et al*. (2000a) and designed primers for 15 microsatellite inserts.

We amplified DNA on a PTC-100 Programmable Thermo Controller (MJ Research Inc.) with 10 µl reaction volumes containing 10 ng genomic DNA, 0.5 µl each of the forward and reverse primers, 5 µl Hotstar Taq Mastermix (Qiagen, Hombrechtikon, Switzerland), and 3 µl of sterilized H_2O. We performed multiplex-PCR with fluorescent labelled primers Poa CA1D4 (label: 6-FAM) and Poa CA1F4 (HEX). We labelled the forward primers at all loci. After a preliminary denaturation step at 95 °C for 15 min., we performed polymerase chain reaction (PCR) amplification as follows: 30 cycles at 95 °C for 30 s, 30 s at locus-specific annealing temperature (Table 1), 30 s at 72 °C, and a final 8 minute extension step at 72 °C. We mixed 1 µl of the PCR product with 10 µl of a 75:1 solution of formamide and GeneScan-500(ROX) size standard (Applied Biosystems, Foster City, USA). We determined fragment lengths on an ABI PRISM 310 Genetic Analyzer using GeneScan 2.1 and Genotyper 2.1 (Applied Biosystems, Foster City, USA). We analyzed all clearly detectable bands after careful verification. Of the 15 microsatellite inserts, five turned out polymorphic among the eight plants (Table 1).

We used these five microsatellite markers to study the genetic diversity of 415 plants of *P. alpina* originating from 54 agriculturally used parcels of land, representing a 170 km gradient across the Swiss Alps. To test whether the number of bands per plant was correlated with the number of chromosomes, we counted chromosomes in the root tips of 25 plants. Plants with more chromosomes had more bands and this relationship was stronger for smaller chromosome numbers (N=25, Linear regression model, linear term t=6.02, p<0.0001, quadratic term t=-6.09, p<0.0001, R^2=0.66). The number of chromosomes per plant was unknown for the other plants, and plants could show more than two bands per locus. Of course, these data do not conform to standard statistics for codominant microsatellite markers of diploid organisms, such as observed and expected heterozygosity and tests for deviation from Hardy-Weinberg equilibrium. Therefore, we prepared a presence-absence matrix for each band across all plants. As measures of genetic diversity per parcel of land, we used mean number of bands per plant and total number of bands per parcel. Moreover, as measure of pairwise genetic distance between parcels of land, we calculated pairwise Euclidian distances based on the relative abundances of each band. Genetic distances between pairs of parcels of land were weakly positively correlated with pairwise geographical distances (r_M=0.12, p=0.057), suggesting that isolation by distance plays a small role in shaping microsatellite diversity of *P. alpina* in the Swiss Alps.

Our results show that a presence-absence matrix of bands allows deriving valuable population genetic information with our markers. Moreover, if ploidy levels are known the markers will also allow determining heterozygosity and F-statistics.

Acknowledgements

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Table 1 Genetic characteristics of 5 *Poa alpina* L. microsatellite loci based on 8 individuals genotyped from one population in the Swiss Alps. The characteristics of repeat motifs are based on the sequenced clones. TA = locus-specific annealing temperature.

Locus	Primer Sequences (5'-3')	Repeat motif	TA [°C]	Size range [bp]	No. of bands	Mean number of bands per plant ± SE	Accession No.
Poa CA1D4	F: AACTTTGGTGCAAGCAGCTC(1)	$(GT)_{14}$ AT $(GT)_2$	60	239-301	12	3.13 ± 0.44	AY942201
	R: ACAGGGAGCTACACAAAGCAG						
Poa GAC1	F: GAAGAACAAGAGGAACAAGATAGC(1)	$(GA)_6$ $(CA)_5(GA)_3$GGGA $(GA)_3$ AA $(GA)_5$G $(GA)_{31}$	60	92-182	20	5.00 ± 0.33	AY942202
	R: AAAAGAGGGCTACGCGATTC						
Poa GA1C3	F: AAGGAGGAACAACTCGAAAGC(1)	$(GA)_{37}$	64	195-293	12	2.00 ± 0.19	AY942203
	R: AGTGTTTCCCTCCTCGATAGTG						
Poa CA1F4	F: ACTGGTTTGTCATCGCACTG(1)	$(GA)_{16}$	60	199-232	12	4.13 ± 0.23	AY942204
	R: TTCCTCATCTAGCGGAGACC						
Poa CAB12	F: TCATCTTGGCCATCATCTTG(1)	$(CA)_{18}$	62	125-172	10	2.63 ± 0.32	AY942205
	R: TTCTGTACGTTGACCTGACTGG						

(1) Fluorescent labelled primer

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5

Microsatellite diversity of the agriculturally important *Poa alpina* L. in the Swiss Alps

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Abstract

Genetic diversity within a species, prerequisite for future evolutionary change, can be shaped by natural and anthropogenic drivers. However, multi-site studies of genetic diversity and the relative importance of their potential abiotic, biotic, and anthropogenic drivers are scarce. We studied genetic diversity in the Alpine Meadow Grass *Poa alpina*, which is common in subalpine and alpine natural sites and on agriculturally used land, where it is an important fodder grass. The species comprises seed-producing and bulbil-producing plants. We assessed genetic diversity with five microsatellite markers for 569 *P. alpina* plants originating from 20 natural sites and 54 grassland parcels of different cultural tradition, land use, and altitude along a 170 km east-west gradient in the Swiss Alps. Low, but significant 1.1 % of the molecular variation in the presence and absence of alleles was between natural and agriculturally used parcels while there was no difference in within-parcel genetic diversity. For agriculturally used parcels, 25.1 % of the molecular variation was among parcels, and 74.9 % within. Of the 25.1 % variation among parcels, 6.8 % were among villages, 4.2 % among parcels with seed-producing and parcels with bulbil-producing plants, and 1.2 % among mown and grazed parcels. Within villages, more different land use types enhanced genetic diversity. Within populations, grazing enhanced genetic diversity compared with mowing. Populations at higher altitudes had higher allelic richness. We conclude that ongoing socio-economically motivated land use changes affect genetic diversity of *P. alpina*, and diverse land use is required to promote high genetic diversity.

Keywords: grassland, cultural tradition, microsatellites, rarefaction, genetic diversity, land use

Introduction

Biodiversity is the product of millions of years of evolutionary processes and a major condition for the long-term viability of ecosystems. The recent interest in the underlying causes of biodiversity has largely concentrated on species diversity, especially of plants (e.g., Proulx & Mazumder 1998; Bruun et al. 2001; Jacquemyn et al. 2003). Although genetic diversity is acknowledged as important level of biodiversity, drivers of genetic diversity, which can be of natural and anthropogenic origin, have received less attention.

High genetic diversity is necessary for populations to adapt to a continuously changing environment (Frankham et al. 2002). Genetic diversity comprises genetic diversity within individuals, genetic differences among individuals within a population, and genetic differences among populations (Meffe & Carroll 1997). Genetic diversity is shaped by the balance between genetic drift, inbreeding, recombination, gene flow, mutation, and selection (Loveless & Hamrick 1984; Hartl & Clark 1997). This balance depends on important life history traits, such as the mode of reproduction or life form (Loveless & Hamrick 1984; Hamrick & Godt 1989; Hamrick & Godt 1997).

Selection can be exerted by both natural and anthropogenic factors. Such potential natural drivers of genetic diversity include abiotic parameters, such as altitude or soil conditions. Moreover, genetic diversity may be affected by the diversity of the surrounding community. Higher plant species richness was suggested to increase genetic diversity, if it increases the diversity of available niches (Odat et al. 2004; Vellend & Geber 2005). Potential anthropogenic drivers of genetic diversity include land use diversity. However, the relative importance of drivers of genetic diversity is not even known for many widespread plant species. Therefore, we studied genetic diversity and its drivers for a common plant which occurs over a large altitudinal range at natural sites and in agriculturally used grassland, the Alpine Meadow grass *Poa alpina* L., in the Swiss Alps.

The species can reproduce via seeds and vegetatively by producing bulbils. In an accompanying common garden study the proportion of genotypes reproducing vegetatively via bulbils was higher among samples from higher altitudes (A. Weyand et al., unpublished), in line with the hypothesis of an adaptive advantage of vegetative reproduction in the harsher conditions at higher altitudes (Bauert 1993; Pluess & Stöcklin 2005; Weppler & Stöcklin 2005). This differentiation in the reproductive mode may affect both within-population diversity and population differentiation. Furthermore, as *P. alpina* occurs across a wide geographical range, isolation by distance is likely to have shaped the distribution of genetic diversity among populations of different regions (Wright 1943). Additionally, divergence

among populations of *P. alpina* could be enhanced due to the known poly- and aneuploidy of the species (Duckert-Henriod & Favarger 1987) which presumably restricts gene flow among individuals and populations (Briggs & Walters 1997).

For about 5000 years the Alpine landscapes and in particular their grasslands have been shaped by human land use (Bätzing 2003). In the European Alps, *P. alpina* is one of the most important fodder grasses for cattle (Conert 1998). Therefore, *P. alpina* has been under agricultural selection pressure for hundreds of years. Plants of *P. alpina* showed adaptation to anthropogenic land use variation in a common garden experiment (A. Weyand et al., unpublished). There, plants from pastures allocated more biomass to reproduction than plants from natural sites did, while plants from meadows allocated less biomass than plants from natural sites did, suggesting divergent selection between parcels of different land use. For the present study, these results matter because higher allocation to reproduction in pastures may suggest that genetic diversity is higher in plants from pastures. Higher genetic diversity in pastures could also be maintained by the spatially more heterogeneous conditions created by grazing animals. In the Alps the relationship between land use and genetic diversity within a species is of particular interest, as due to land use changes during the last decades many meadows have been converted to pastures (Bätzing 2003).

In the Swiss Alps, the cultural traditions Romanic, Germanic, and Walser contributed to a high landscape diversity through their different agricultural practices (Bätzing 2003). If differences in land use lead to genetic divergence between plants, villages with higher land use diversity may harbour higher genetic diversity of *P. alpina* than villages with lower land use diversity.

We studied the effects of natural factors and agricultural land use on genetic diversity of *P. alpina* within and among 12 villages in the Swiss Alps. Each of the three cultural traditions Romanic, Germanic, and Walser was represented by four villages. At the parcel level we studied genetic diversity within and among populations from 20 natural sites and from 54 agriculturally used grasslands at different altitudes in these 12 villages. The agriculturally used parcels were either mown or grazed and they were either additionally fertilized or unfertilized. Plant species diversity was known for all parcels from a previous study (K. Maurer et al., unpublished).

As molecular markers we used five polymorphic microsatellite loci (Maurer et al. 2005). Microsatellites are generally assumed to be selectively neutral (Scribner & Pearce 2000) and they offer high resolution (Schlötterer 1998). Therefore, they are ideal to investigate gene flow and genetic drift. Even though natural selection is highly unlikely to act on the

investigated microsatellite loci themselves, it would nevertheless affect their diversity because of linkage between microsatellite loci and loci under selection (Till-Bottraud & Gaudeul 2002).

We asked the following questions: (1) Are *Poa alpina* populations from agriculturally used grassland genetically differentiated from natural populations? (2) Is genetic differentiation among villages and among populations related to geographical distances, to differences in land use, and to differences in reproductive modes? (3) Is genetic diversity within villages related to cultural traditions and to land use diversity? (4) Is genetic diversity within grassland parcels related to altitude, land use, and reproductive mode?

Methods

Study species

Poa alpina L. (Poaceae) is a common grass at subalpine and alpine levels in the northern hemisphere. Its presence indicates high levels of nutrients and soil moisture (Conert 1998). Accordingly, it occurs in pastures and nutrient rich meadows, but also as a pioneer species in scree slopes and in snowbeds. In the European Alps, *P. alpina* is among the most important fodder grasses due to its high contents of fats and proteins (Bachmann 1980; Conert 1998). The species constitutes a polyploid complex with common aneuploidy (Müntzing 1980) and highly variable chromosome numbers (Steiner & Heidenreich 1997). In Switzerland reported chromosome numbers range from 2n=22 to 46 (Duckert-Henriod & Favarger 1987), and more than 60 chromosomes were found in Scotch plants (Müntzing 1980).

Some plants of *P. alpina* produce seeds, while others reproduce vegetatively. The latter form bulbils in the panicles instead of seeds (Müntzing 1980). Such bulbils grow into little plantlets on the maternal plants, which therefore are called pseudoviviparous. Eventually, the plantlets may dehisce from the maternal plant and root (Pierce 1998). Usually, pseudoviviparous plants also develop a sexual floret at the basis of the plantlets (Philipson 1934; Müntzing 1980; Pierce et al. 2003). It is not known whether these sexual florets produce fertile pollen and viable seeds and whether there is gene flow between such florets and sexually reproducing plants. The mode of reproduction appears largely genetically determined, while phenotypic plasticity in the mode of reproduction plays a minor role (Schwarzenbach 1953; Schwarzenbach 1956; Heide 1989).

Study area

The study area comprises 12 villages in the Swiss Alps, four of each of the three cultural traditions Romanic, Germanic, and Walser (Fig. 1). The villages are situated along a 170 km long east-west gradient. Each village belongs to a separate alpine valley. To represent typical agricultural villages, the study villages were randomly selected with the restriction that their agricultural character has only changed modestly during the last 50 years, and that they are not very touristy and do not have more than about 1500 inhabitants.

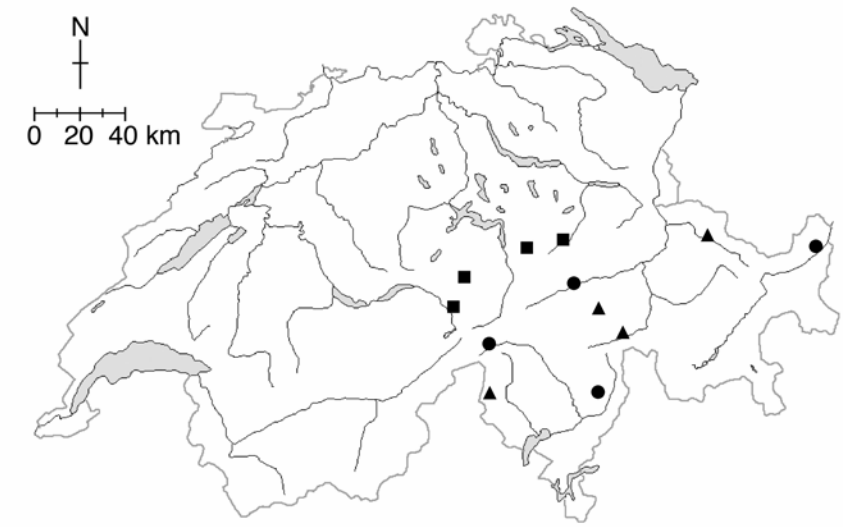

Figure 1 Map of Switzerland with the 12 study villages and their cultural traditions.
● = Romanic, ■ = Germanic, ▲ = Walser

Study design and sampling

We searched for *P. alpina* in parcels of land chosen for a vegetational survey of grasslands (K. Maurer et al., unpublished). In each village these grassland parcels were situated at three altitudinal levels, at the valley bottom (about 1000 m asl), at intermediate altitudes (about 1500 m asl), and at the alp level (about 2000 m asl). We searched for *P. alpina* L. in those of 216 grassland parcels where, according to local farmers, the type of land use had always been the same. These parcels were either mown or grazed and were either additionally fertilised with dung or liquid manure or were unfertilised. In each parcel with *P. alpina*, we sampled eight plants at interdistances of five meters. Altogether, we sampled plants from 54 agriculturally used grassland parcels, 13 meadows and 41 pastures, of which 19 were additionally fertilised and 35 were not. Moreover, in the same way we sampled plants from 20 natural sites above tree line that had never been used agriculturally. Finally, we analysed 415 plants from agriculturally used parcels and 154 plants from natural sites, between six and eight plants per parcel.

Microsatellite analysis

We dried collected leaf samples immediately with silica gel. Then, we ground about 30 mg of the material in Eppendorf tubes with a glass bead in a shaking mill. We extracted DNA according to a Rogers & Bendich (1994) protocol modified by Steinger et al. (1996), except that we incubated samples with CTAB buffer and mercaptoethanol at 65° C.

We screened all plants for variation at five polymorphic microsatellite loci (Maurer et al. 2005). We amplified DNA with 10 µl reaction volumes containing 10 ng genomic DNA, 0.5 µl each of the fluorescence-labelled forward primer and of the reverse primer, 5 µl Hotstar Taq Mastermix (Qiagen, Hombrechtikon, Switzerland), and 3 µl of sterilized H2O. After a preliminary denaturation step at 95° C for 15 min., we amplified DNA with polymerase chain reaction (PCR) on a PTC-100 Programmable Thermo Controller (MJ Research Inc.) for 30 cycles of 30 s denaturing at 95° C, 30 s of annealing at locus-specific temperatures (Maurer et al. 2005) and 30 s of extension at 72° C, with a final 8 minute extension step at 72° C. We mixed 1 µl of the PCR product with 10 µl of a 75:1 solution of formamide and GeneScan-500(ROX) size standard (Applied Biosystems, Foster City, CA, USA). We determined allele lengths with an ABI PRISM 310 Genetic Analyzer using GeneScan 2.1 and Genotyper 2.1 (Applied Biosystems, Foster City, CA, USA).

Analysis of differences among villages and among grassland parcels

To test for differentiation between natural and agricultural sites we partitioned molecular variation between natural sites and agriculturally used parcels, among parcels, and within parcels with analysis of molecular variance (AMOVA, Excoffier et al. 1992) based on the pairwise Euclidian distance matrix of the presence and absence of alleles in individuals of all 74 parcels. Then, to test whether regional differentiation was similar for natural and agricultural sites we partitioned molecular variation among villages, among parcels, and within parcels separately for the plants of the 54 agriculturally used parcels and for those of the 20 natural sites.

Moreover, to examine potential differentiation among parcels of grassland of different land use (mown versus grazed) and fertilisation (fertilised versus unfertilised), we also partitioned molecular variation among these groups, among parcels within these groups, and within parcels with AMOVA including plants of the 54 agriculturally used parcels. Furthermore, we used AMOVA to examine potential differentiation between 10 parcels from which all sampled plants exclusively reproduced pseudoviviparously in the common garden

(A. Weyand et al., unpublished), and 22 parcels from which all sampled plants exclusively produced seeds.

To identify potential determinants of genetic differentiation among villages, we used Mantel tests (Mantel 1967; Manly 1997). We tested the relationship of the matrix of Euclidean genetic distances among villages, based on the relative abundance of each allele occurring in agriculturally used parcels per village, with the matrix of geographical distances. Moreover, we tested the relationship of the matrix of genetic distances and the matrix of cultural distances, where pairs of villages of the same cultural tradition were assigned the cultural distance 0, and pairs of villages of different traditions 1, with a Partial Mantel test (Legendre & Legendre 1998, n = 1000 permutations) where we controlled for geographical distances.

Additional to AMOVA we measured among-parcel differentiation as differentiation for allelic richness $\rho_{ST(n)}$ among all 54 agriculturally used populations and also among meadows and pastures separately following El Mousadik & Petit (1996) and Petit *et al.* (1998). To obtain $\rho_{ST(n)}$, first we calculated the expected allelic richness $r'_{T(n)}$ of a random sample of n=6 plants out of all 415 plants and $r'_{S(n)}$ of a random sample of n=6 plants for each parcel. Then, we calculated the differentiation for allelic richness as $\rho_{ST(n)} = 1 - r'_{S(n)} / r'_{T(n)}$ (where S represents the single parcels and T the total population of all sampled plants) for each locus separately and the mean across the five loci.

To identify potential determinants of genetic differentiation among agriculturally used parcels we used Mantel tests (Mantel 1967; Manly 1997). We tested the relationship of the matrix of Euclidean genetic distances between parcels, based on the relative abundance of each allele per parcel, with the matrix of geographical distances among parcels. Furthermore, we obtained the matrix of altitudinal differences among parcels, the matrix of land use distances, where pairs of parcels of the same land use (mowing or grazing) were assigned the land use distance 0, and pairs of parcels of different land use 1, and similarly, the matrix of fertilisation distances (fertilised vs. unfertilised). Controlling for effects of geographic distance we did Partial Mantel tests (Legendre & Legendre 1998, n = 1000 permutations) of the relationships between the matrix of genetic distances and these other matrices. Similarly, we tested the relationship between genetic distance among parcels and distances in reproductive mode with a partial Mantel test controlling for geographical distance considering the 32 grassland parcels with samples with a uniform reproductive mode as described above.

In the matrix of distances in reproductive mode, pairs of parcels of the same reproductive mode were assigned the value 0, pairs with different modes the value 1.

Analysis of genetic diversity within villages and within parcels

We measured genetic diversity within villages and within parcels as allelic richness $r'_{(n)}$ for each locus following El Mousadik & Petit (1996), except that we used plants as sample units instead of genes. We used the rarefaction procedure of Hurlbert (1971) to estimate allelic richness for a standardized sample size of n plants. As rarefaction sample size we used the smallest one available, $n = 16$ for villages, and $n = 6$ for parcels. For each locus, according to the formula $\hat{r}_{(n)} = \sum_i \left[1 - \binom{N - N_i}{n} \Big/ \binom{N}{n} \right]$ (where N_i represents the number of occurrences of the i^{th} allele among the N sampled plants of a population and n the standardized sample size), we calculated the expected number of different alleles $r_{(n)}$ in a sample of n plants. We obtained the corrected allelic richness $r'_{(n)}$ by subtracting one from the allelic richness $r_{(n)}$, because a village or a parcel with only one single allele is considered to be monomorphic. Then we calculated mean allelic richness over all five loci. For simplicity we further on call the latter allelic richness.

We tested potential effects of the village characteristics latitude and longitude, ratio of the numbers of meadows and pastures in a village, altitude, number of land use combinations (combination of land use and fertilisation) investigated in a village, and culture on allelic richness per village with analysis of variance (ANOVA). For these analyses, only plants from agriculturally used parcels in each village were included.

To analyse within-parcel genetic diversity, we investigated effects of cultural tradition, altitude, land use, and fertilisation on allelic richness per parcel and on the mean number of alleles per plant for each parcel with hierarchical analysis of covariance (ANCOVA) with sequential sums of squares. We tested effects of culture against remaining variation among villages and of all other factors against variation due to remaining differences among parcels. To account for differences among parcels because of different soil conditions or solar radiation, we used pH values and aspect of each parcel as covariates. However, as these covariates did not qualitatively change the results, we present results without covariates. We also tested whether there was a difference in within-parcel diversity between populations from

agriculturally used parcels and populations from natural sites. As there was no difference, we present results including only the agriculturally used parcels.

Moreover, we calculated one-way ANOVAs to test potential effects of reproductive modes on the measures of within-parcel genetic diversity. In addition to the parcels with exclusively pseudoviviparous or seed producing samples, we also considered 21 parcels with samples of *P. alpina* containing plants of both reproductive modes.

We did all statistical analyses with the software R (R Development Core Team 2004). For Mantel tests and Partial Mantel tests we used the R-package vegan (Oksanen 2005) and for AMOVAs the R-package ade4 (Thioulouse *et al.* 2004).

Results

Overall microsatellite diversity and differentiation between natural and agriculturally used grassland parcels

Among the 569 plants of *Poa alpina* we altogether detected 209 alleles at the five microsatellite loci, between 25 and 61 per locus. In total we detected 531 multilocus-microsatellite phenotypes among all 569 plants and 386 multi-locus microsatellite phenotypes among the 415 plants from agriculturally used parcels.

Low, but highly significant 1.1 % of the variation in the presence and absence of microsatellite alleles resided between natural and agriculturally used grassland parcels (AMOVA, $P < 0.004$).

Genetic diversity among villages

8.4 % and 6.8 % of the variation in the presence and absence of alleles resided between villages considering individuals from natural or agriculturally used sites, respectively (AMOVA, for both $P < 0.001$, Table 1). Genetic distances between villages were independent of geographic distances (Mantel test with plants from agriculturally used parcels, $r_M = 0.22$, $P = 0.16$) and cultural differences (Partial Mantel test with plants from agriculturally used parcels and with geographic distance as covariate, $r_M = 0.02$, $P = 0.28$) among pairs of the 12 villages.

Table 1 Summary of analysis of molecular variance (AMOVA) of microsatellite phenotypes of plants of *Poa alpina* from 54 agriculturally used parcels and from 20 natural sites grouped in 12 villages. AMOVA was based on the matrix of pairwise Euclidian distance between individuals in the presence and absence of microsatellite alleles.

		Variance component		
Source of variation	d.f.	Absolute	%	*P*
54 populations from agriculturally used parcels				
Among villages	11	0.7981	6.76	< 0.001
Among parcels within villages	42	2.1790	18.39	< 0.001
Within parcels	361	8.8422	74.86	< 0.001
Total	414	11.8122	100.00	
20 populations from natural sites				
Among villages	10	1.0184	8.42	< 0.001
Among parcels within villages	9	2.2502	18.61	< 0.001
Within parcels	134	8.8203	72.96	< 0.001
Total	135	12.0889	100.00	

Genetic diversity within villages

Allelic richness per village, based on a standardized sample size of 16 plants, was between 11.2 and 16.6 alleles, with a mean of 14.5. Allelic richness was higher in villages with higher numbers of land use combinations among the sampled parcels ($P < 0.05$, Fig. 2 a). In villages with Walser tradition, allelic richness was significantly higher than in Germanic villages (Tukey HSD, $P < 0.05$, Fig. 2 b). When both these significant variables were fitted simultaneously, the effect of cultural tradition only was significant when introduced into the model before the number of land use combinations (introduced first: $P < 0.05$, second: $P = 0.15$), while the significant effect of the number of land use combinations was independent of the fitting sequence.

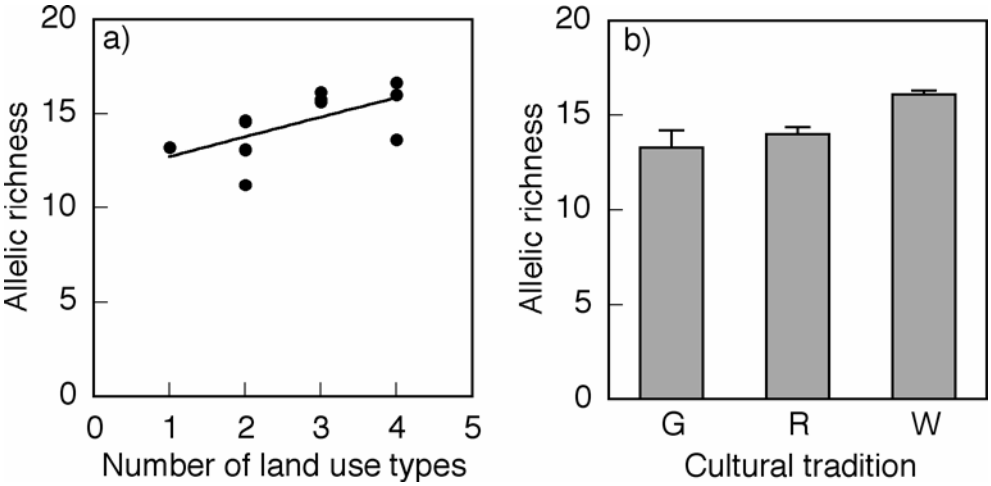

Figure 2 Relationship between microsatellite allelic richness of *Poa alpina* and a) number of land use types investigated per village and b) cultural tradition of 12 villages in the Swiss Alps. Allelic richness is based on a standardized sample size of 16 plants. G = Germanic tradition, R = Romanic, W = Walser. In Fig. 2a three data points are hidden by others.

Genetic diversity among agriculturally used parcels

25.1 % of the variation in the presence and absence of alleles resided among parcels (AMOVA, $p < 0.001$) and 18.4% resided among parcels within villages (AMOVA; $p < 0.001$, Table 1). Parcel differentiation for allelic richness $\rho_{ST(n)}$ was 0.25 across all five loci (Table 2), in line with the AMOVA result. Differentiation among the 13 meadows and among the 41 pastures was 0.32 and 0.23, respectively (Table 2).

Geographically more distant parcels tended to be genetically more distant (Mantel test, $r_M = 0.12$, $P = 0.057$, Fig. 3 a), while genetic distances were independent of altitudinal differences among parcels (Partial Mantel test with geographic distance as covariate, $r_M = -0.03$, $P = 0.65$). Samples of *P. alpina* from parcels of the same land use (mowing or grazing) were genetically more similar than those from parcels with different land use (Partial Mantel test, $r_M = 0.15$, $P < 0.05$, Fig. 3 b), while fertilisation did not affect genetic diversity among parcels ((Partial Mantel test, $r_M = 0.07$, $P = 0.095$). Accordingly, when we partitioned variation between parcels according to

Table 2 Population genetic measures for 54 populations from agriculturally used parcels of grassland in the Swiss Alps.

Microsatellite locus	Mean number of alleles per plant and population	$r'_{S(6)}$	$r'_{T(6)}$	$\rho_{ST(6)}$	$\rho_{ST(6)}$ among meadows	$\rho_{ST(6)}$ among pastures
Poa CA1D4	2.88	7.19	9.03	0.20	0.29	0.18
Poa GAC1	4.44	11.62	16.35	0.29	0.36	0.27
Poa GA1C3	2.23	5.12	6.75	0.24	0.34	0.21
Poa CA1F4	3.87	8.39	10.57	0.21	0.25	0.19
Poa CAB12	3.37	7.29	10.33	0.29	0.34	0.28
Mean	**3.36**	**7.92**	**10.61**	**0.25**	**0.32**	**0.23**

$r'_{S(6)}$ = Allelic richness per population with a standardized sample size of six plants
$r'_{T(6)}$ = Allelic richness of the hypothetical total population with a sample size of six plants
$\rho_{ST(6)}$ = Differentiation for allelic richness among populations
$\rho_{ST(6)}$ among meadows = Differentiation for allelic richness among 13 meadows
$\rho_{ST(6)}$ among = Differentiation for allelic richness among 41 pastures

land use, we found 1.18 % to reside between mown and grazed grassland parcels (AMOVA, P < 0.02), and 0.02 % between fertilised and unfertilised grassland parcels (AMOVA, P > 0.42).

Genetic distances between samples of parcels with different modes of reproduction were significantly larger than between samples of parcels with the same mode of reproduction (Partial Mantel test, r_M = 0.17, P < 0.05, Fig. 3 c). Accordingly, 4.2 % of the variation resided between the 22 parcels with exclusively seed producing samples of *P. alpina* and the 10 parcels with exclusively pseudoviviparous ones (AMOVA, p < 0.001).

Overall, the results of this section indicate substantial genetic differentiation of *P. alpina* among parcels of different villages and among parcels with different reproductive modes of *P. alpina*. Genetic differentiation among mown and grazed parcels was less pronounced.

Genetic diversity within agriculturally used parcels

74.9 % of the variation in the presence and absence of alleles resided within parcels (AMOVA; p < 0.001; Table 1). Allelic richness, based on a sample size of six plants, varied between 5.12 and 11.62 per parcel and locus (Table 2). The mean number of alleles was between 2.88 and 4.44 per plant and locus (Table 2), and across all loci it was 16.8 per plant. Allelic richness increased with increasing parcel altitude (Fig. 4), while the mean number of alleles per plant was independent of altitude (Table 3). Cultural traditions did not affect genetic diversity within parcels (Table 3).

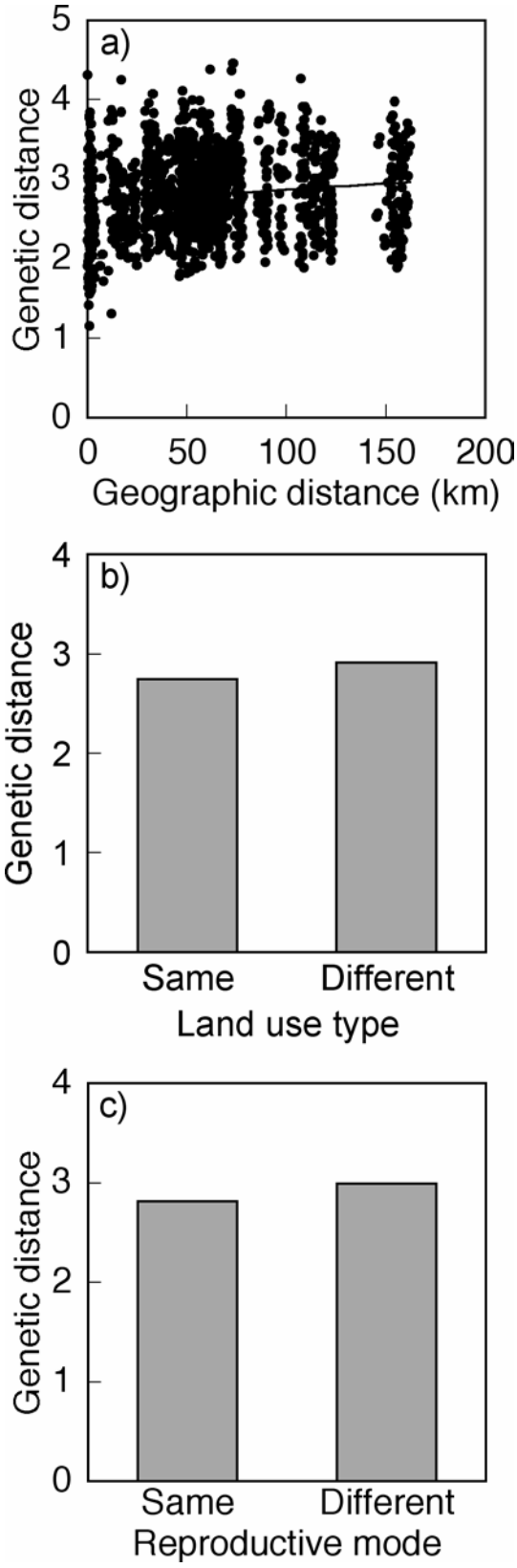

Figure 3 Relationships of pairwise Euclidean genetic distances between populations of *Poa alpina* from agriculturally used parcels of grassland in the Swiss Alps a) with pairwise geographic distances for all 54 parcels, b) with pairwise correspondence or difference in land use for the 41 pastures and 13 meadows, and c) with pairwise correspondence or difference in reproductive mode for 10 parcels with exclusively pseudoviviparously reproducing samples of *Poa alpina* and 22 exclusively seed-producing samples.

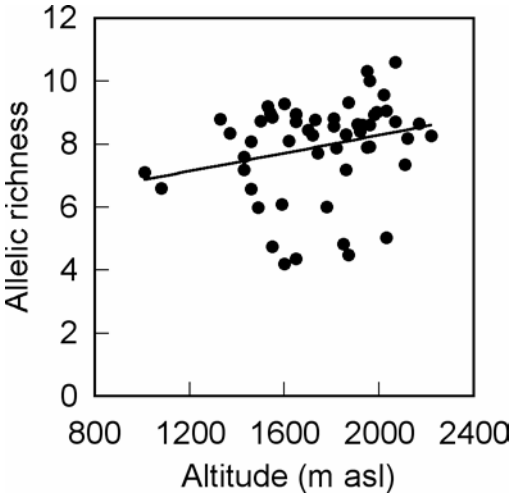

Figure 4 Relationship between microsatellite allelic richness, based on a sample size of six plants, and altitude of 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps.

Table 3 Summary of analyses of the mean number of microsatellite alleles per plant (MA) and allelic richness (AR) in 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps. Culture denotes the Romanic, Germanic, and Walser cultural traditions. Village denotes the 12 study villages. Fertilisation denotes the difference between unfertilised and fertilised parcels. Land use denotes differences between mown and grazed grasslands. In the sequential sums of squares ANCOVA, effects of culture were tested against remaining variation among villages. n.s. denotes values of $p > 0.1$

Source of variation	df	SS_{MA}	F_{MA}	p_{MA}	SS_{AR}	F_{AR}	p_{AR}
Culture	2	4.34	0.24	n.s.	7.72	2.04	n.s.
Village[Culture]	9	81.38	7.54	p<0.01	17.01	1.12	n.s.
Altitude	1	3.94	3.28	p<0.1	9.60	5.67	p<0.05
Fertilisation	1	0.25	0.21	n.s.	0.05	0.03	n.s.
Land use	1	8.78	7.32	p<0.05	6.83	4.04	p<0.1
Fertilisation*land use	1	0.60	0.50	n.s.	0.55	0.33	n.s.
Culture*altitude	2	4.00	1.00	n.s.	1.45	0.31	n.s.
Village[Culture]*altitude	9	18.05	1.67	n.s.	20.80	1.37	n.s.
Culture*fertilisation	2	1.34	0.39	n.s.	3.90	1.16	n.s.
Culture*land use	2	5.47	1.16	n.s.	0.98	0.08	n.s.
Village[Culture]*fertilisation	7	12.12	1.44	n.s.	11.78	0.99	n.s.
Village[Culture]*land use	3	7.09	1.97	n.s.	18.10	3.57	p<0.1
Altitude*fertilisation	1	0.11	0.09	n.s.	0.00	0.00	n.s.
Altitude*land use	1	0.40	0.33	n.s.	6.26	3.70	p<0.1
Residuals	11	13.19			18.61		

The mean number of alleles per plant was 3.0 % higher in pastures than in meadows ($F_{1,53}$ = 7.32; $P < 0.05$, Table 3, Fig. 5). Moreover, allelic richness was marginally significantly higher in pasture parcels than in meadow parcels ($F_{1,53} = 4.04$; $P = 0.07$, Table 3). The mean number of alleles per plant was non-significantly higher for parcels with exclusively seed-producing samples than for those with exclusively or partially pseudoviviparous samples ($F_{2,51} = 1.19$, $P = 0.31$, Fig. 6a). Allelic richness was similar for parcels with exclusively seed-producing or exclusively pseudoviviparous samples, and non-significantly higher in parcels with samples containing plants of both reproductive modes ($F_{2,51} = 2.23$, $P = 0.12$, Fig. 6b).

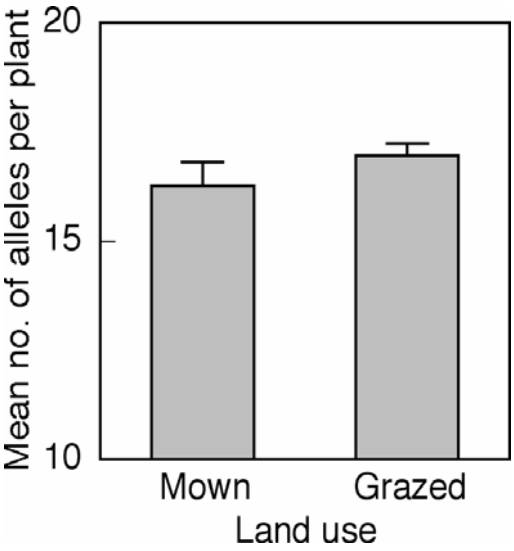

Figure 5 Relationship between number of alleles per plant and type of land use among 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps.

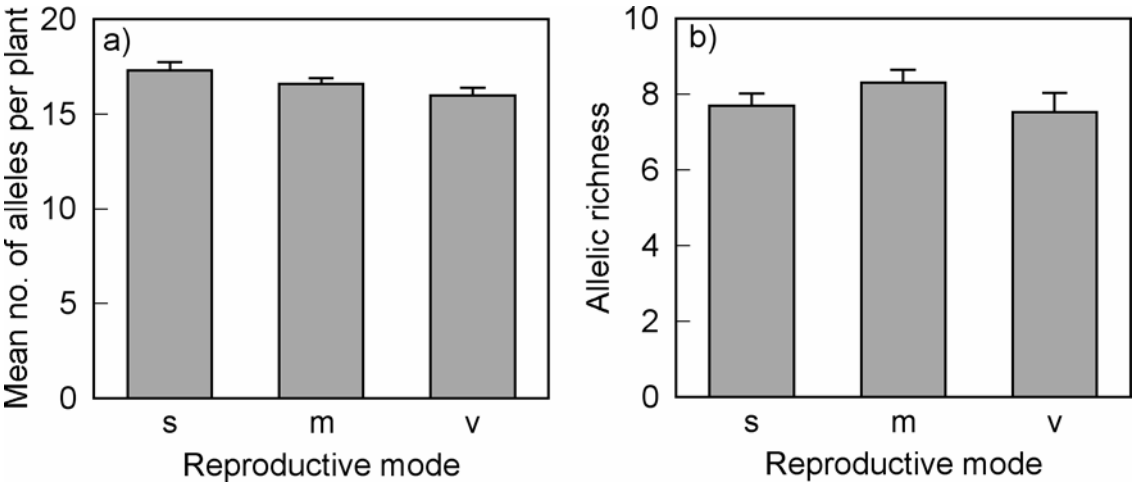

Figure 6 Relationship between a) the mean number of microsatellite alleles per plant and b) allelic richness and the reproductive modes of samples of 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps. s denotes exclusively seed-producing parcel samples, v exclusively pseudoviviparous ones, and m mixed ones.

Mean number of alleles and allelic richness per parcel (Spearman's rank correlation, $p = 0.3714$ and $p = 0.6486$, respectively) and mean Euclidean genetic distance of each parcel to all other parcels (Spearman's rank correlation, $p = 0.6599$) were independent of mean abundance of *P. alpina*. This indicates that microsatellite diversity was not affected by more pronounced genetic drift in smaller populations with lower abundance of *P. alpina*.

Correlations between species richness in two 5 m x 5 m plots per parcel and the mean number of alleles or allelic richness explained between 0.03 and 4 % of the variation and were not statistically significant, indicating that genetic diversity of *P. alpina* was not affected by the species diversity of the 54 agricultural grassland parcels.

Discussion

Genetic differentiation between natural and agriculturally used parcels

The small but highly significant microsatellite differentiation between natural sites and agriculturally used parcels suggests that land use not only caused a divergence in genetic diversity between populations of *P. alpina* from mown and grazed grasslands, but also differentiation from natural habitats. This is in line with an accompanying quantitative genetic experiment where plants from pastures and from meadows both differed from plants from natural sites in biomass allocation to reproduction (A. Weyand et al., unpublished). To our knowledge only one study considering populations from natural sites and different agriculturally used parcels of land has been done, which investigated *Sesleria albicans* (Reisch et al. 2003). Unfortunately, the authors did not separate natural from agricultural populations but only analysed whether there was general variation in genetic diversity among habitat types, which impedes a comparison with their results.

Genetic differentiation among villages and among populations

We studied plants of *P. alpina* from a 170 km range comprising different alpine valleys. Accordingly, genetic differentiation between grasslands was pronounced and explained 25% of the variation, and isolation by distance was apparent as marginally significantly positive relationship between pairwise geographical and genetic distances among parcels. Previously, in *P. alpina* isolation by distance had been studied and observed only within a pseudoviviparous population in Norway (Bjørnstad et al. 1995). In addition to geographic distance, polyploidy, which can restrict gene flow among individuals (Briggs & Walters 1997), may have contributed to population differentiation in *P. alpina* which constitutes a

highly polyploid complex with common aneuploidy (Müntzing 1980; Duckert-Henriod & Favarger 1987; Maurer et al. 2005).

Differentiation due to land use

Different habitat conditions can lead to genetically based ecotypic differentiation in grass species (Stapledon 1928). In our study, mowing and grazing over hundreds of years apparently led to genetic differentiation between mown and grazed populations of *P. alpina* while fertilisation had no effect. This corresponds with the results of a common garden experiment using the same genotypes, which showed a divergence in biomass allocation between plants from mown and grazed parcels, but no difference between plants from fertilised and unfertilised parcels (A. Weyand et al., unpublished). Although the effect of mowing and grazing on genetic divergence between parcels was smaller than effects of isolation by distance or reproductive mode, it adds to the evidence that land use not only affects biodiversity at the community level, but also at the level of selectively neutral molecular diversity within species (Odat et al. 2004). The result is especially remarkable as it suggests that land use affects biodiversity independently of regional differences. Land-use induced ecotypes have also been reported for the grassland forbs *Rhinanthus alectorolophus* (Zopfi 1993) and *Euphrasia rostkoviana* (Zopfi 1998).

The observed population differentiation was higher among populations of meadows than among populations of pastures (Table 2). There are two mutually not exclusive explanations for this result. Firstly, there could be higher gene flow among pastures due to endozoochorous or exozoochorous seed transport by cattle. Secondly, the result could indicate more diverse habitat conditions among meadows than among pastures. Land use intensity of the investigated meadows with *P. alpina* varies between mowing every second year to twice per season. Different mowing intensity was reported to exert differential selection in *Festuca pratensis* (Kölliker et al. 1998), and may thus be responsible for differentiation among mown parcels. Among pastures land use intensity rather varies between seasons than between parcels, which results in more uniform selection, and may thus contribute to the weaker genetic differentiation among pastures than among meadows. A third alternative explanation of higher genetic drift among meadows than among pastures can be ruled out because abundance of *P. alpina* was neither correlated with within-parcel genetic diversity nor with genetic distance of a parcel to all other parcels.

Differentiation due to reproductive mode

Our results revealed substantial genetic differentiation among parcels with exclusively seed-producing samples of *P. alpina* and those with exclusively pseudoviviparous ones (AMOVA and Mantel test). This suggests, that gene flow between seed-producing plants of *P. alpina* and pseudoviviparous ones, which produce a sexual floret at the basis of their inflorescence (Philipson 1934; Müntzing 1980), is either very low or even absent.

Genetic diversity within villages

The statistical significance of the effect of cultural tradition on allelic richness of *P. alpina* within villages depended on the fitting sequence. Therefore, this effect appears to have been mediated by the different number of different land use types of investigated parcels with *P. alpina* in villages of different cultural tradition. In Walser villages more different land use combinations with *P. alpina* tended to be present than in Romanic and Germanic villages (result not shown). Most likely, this is due to the combination of the alpine to subalpine altitudinal distribution of *P. alpina* with the higher altitudes of Walser villages than of villages of the other traditions. The latter is due to settlement history, where the later arriving Walser people had to settle at higher altitudes than the Romanic and Germanic people (Bätzing 2003). Accordingly, at the valley bottom of villages at lower altitudes *P. alpina* was not present in all types of parcels, which reduced the number of investigated land use combinations in Romanic and Germanic villages. The higher genetic diversity of *P. alpina* in villages where *P. alpina* occurred in a larger number of different land use combinations is in line with the observed microsatellite differentiation among parcels of different land use. Moreover, it corresponds to the result of a study of plant species diversity of the same 12 villages, which revealed a significantly positive relationship between plant species richness per village and the number of different land use combinations present in the villages (K. Maurer et al., unpublished).

Genetic diversity within populations

Agricultural land use significantly affected within-population genetic diversity of *P. alpina*. Populations originating from pastures were genetically more diverse with significantly more alleles per plant and marginally significantly greater allelic richness than meadow populations. Because samples of *P. alpina* from meadows and those from pastures did not differ significantly in their reproductive modes (A. Weyand et al., unpublished), variation in reproductive mode cannot be responsible for these effects of land use on genetic diversity.

Rather, this may be due to higher recruitment in grazed sites, either because of the higher biomass allocation of plants from pastures to reproduction (A. Weyand et al., unpublished), or because of the higher probability of establishment of seedlings and pseudoviviparous plantlets in pastures, which offer more vegetation gaps as safe sites for establishment (Grubb 1977). Moreover, selection in mown sites may be more uniform than in spatially more heterogeneous grazed sites, which may reduce genetic diversity more strongly in meadows than in pastures. Accordingly, in *Festuca pratensis* molecular genetic diversity was the lower, the more intense the cutting regime was (Kölliker et al. 1998). Furthermore, genetic diversity could have been enhanced in pastures because of higher gene flow due to seed transport by cattle. In contrast to our findings with *P. alpina*, genetic diversity of *F. pratensis* populations was also affected by fertilisation (Kölliker et al. 1998).

Independent of land use effects, variation in reproductive mode also affected genetic diversity within parcels. In line with expectations based on recombination (Hartl & Clark 1997), plants from parcels with exclusively seed-producing samples had non-significantly more alleles than the intermediate number detected in plants from parcels with mixed samples, and the lowest number in parcels with exclusively pseudoviviparous samples. Allelic richness was non-significantly higher in mixed than in pseudoviviparous and seed-producing samples. This corresponds well with the observed genetic differentiation between parcels with seed producing and pseudoviviparous samples (AMOVA and Mantel test).

Parcels from higher altitudes had higher allelic richness. This may well be related to the differentiation between pseudoviviparous and seed-producing samples of *P. alpina* (Fig. 3 c) in combination with an increase in pseudoviviparous reproduction from 20 % in valley genotypes to 50 % in alpine genotypes observed in the common garden (A,. Weyand et al., unpublished). The number of alleles per plant, which was not significantly different between exclusively pseudoviviparous and exclusively seed-producing samples, did not increase with altitude.

The independence of microsatellite diversity of *P. alpina* from plant species diversity does not support the hypothesis that niche diversity is higher in more species rich grasslands, which was suggested to maintain higher genetic diversity (Odat et al. 2004; Vellend & Geber 2005). In our study, structural heterogeneity was higher in pastures than in meadows which was also reflected by the higher genetic diversity in populations from pastures, but this was independent from species diversity.

Conclusions

Genetic diversity of *P. alpina* turned out to be affected by the natural altitudinal gradient, by land use diversity in villages, and by specific land use within parcels. Higher genetic diversity within pastures than within meadows, genetic differentiation between populations from meadows and pastures, and higher genetic diversity within villages with more diverse land use imply two important conclusions. First, they demonstrate that the ongoing socio-economically motivated land use changes in the Swiss Alps do not only affect biodiversity at the landscape and community levels, but also change biodiversity within species. Moreover, promoting genetic diversity cannot be achieved by just maintaining the single type of land use associated with highest within-population diversity, but requires the maintenance of a high diversity of land use types.

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6

Biotic and abiotic determinants of quantitative genetic variation of the common grass *Poa alpina* L. in grasslands in the Swiss Alps

(submitted)

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Abstract

Multi-site studies of heritabilities and their potential drivers are very scarce. We studied broad-sense heritability H^2 in an important fodder grass, the clonal *Poa alpina*. We estimated H^2 of several reproductive and vegetative characters in a common garden experiment with 825 *P. alpina* plants originating from 53 grassland parcels of different cultural tradition, land use, and altitude along a 170 km east-west stretch in the Swiss Alps. Estimates of H^2 were significant for all traits and ranged from 0.363 to 0.572. H^2s were larger for parcels more rich in potassium, and twice as high for grazed than for mown ones. Moreover, and most likely associated with landscape diversity, H^2s were higher for parcels from villages of the old Romanic cultural tradition than for those of the Germanic and Walser traditions. Overall, land use was the most important determinant of H^2. We suggest promoting diverse land use regimes to conserve not only landscape and plant species diversity, but also heritable genetic diversity of *P. alpina*.

Key words: intraspecific diversity, broad-sense heritability, genetic variation, biodiversity, land use, agricultural land use, conservation

Introduction

The heritable genetic component of phenotypic variation is prerequisite for evolution and adaptation (Stearns, 1992). Potential natural drivers of heritable variation include abiotic parameters, such as topography and soil conditions, and biotic parameters, such as plant species diversity and the diversity of biological interactions. Potential anthropogenic drivers of heritable variation include variation in land use. However, heritable variation and the relative importance of its drivers are not even known for many widespread plant species. Therefore, we assessed heritable genetic variation and its drivers for the common Alpine Meadow grass *Poa alpina*, which in the Alps occurs over a large altitudinal range at natural sites and in agriculturally used grassland, where it is among the most important fodder species (Conert, 1998).

Heritability can be assessed in several ways. Narrow-sense heritability, which measures the proportion of additive genetic variation relative to total phenotypic variation (Falconer & MacKay, 1996), is considered the best predictor of response to selection for sexually reproducing plants. Broad-sense heritability measures the proportion of genotypic variation relative to total phenotypic variation (Falconer & MacKay, 1996). In sexually reproducing organisms it generally overestimates realized response to selection, because variation between genotypes, even in the absence of maternal carry-over effects, may not only include additive genetic variation, but also variation due to dominance or epistasis (Stearns, 1992). However, in clonal plants vegetative reproduction contributes largely to offspring reproduction, and therefore broad-sense heritability constitutes a better estimate for realized response to selection in clonal plants than in exclusively sexually reproducing plants (Fischer et al., 2004). A powerful tool to partition phenotypic variation into genotypic and environmental components is provided by experimentation in the common garden, where plant growth and plant response to simulated land use can be studied under uniform conditions (Silvertown & Charlesworth, 2001).

Heritable genetic variation is especially important for traits closely associated with fitness. Therefore, we studied heritable variation in growth and reproduction. Under stabilizing selection, such traits are likely to have low heritabilities (Stearns, 1980). Nevertheless, in heterogeneous environments substantial heritabilities of fitness-relevant traits may be maintained.

The landscapes of the Alps have been co-shaped by humans and their cattle over thousands of years (Ellenberg, 1996). Therefore, in the cultural landscape of the Alps, *P. alpina* has been under agricultural selection pressure for hundreds of years. In an

accompanying study, we found *P. alpina* to be adapted to the natural altitudinal gradient (Chapter 3). While plants originating from different altitudes did not differ in vegetative growth, the proportion of genotypes reproducing vegetatively via bulbils rather than via seed was higher among genotypes from higher altitudes, in line with the hypothesis of adaptive advantage of vegetative reproduction in the harsher conditions at higher altitudes (Klimeš et al., 1997). Moreover, plants of *P. alpina* turned out to be adapted to anthropogenic land use variation (Chapter 3). In the common garden, plants from meadows had higher vegetative growth than plants from pastures, while plants from pastures allocated more biomass to reproduction than plants from meadows did. Plants from natural sites were intermediate, suggesting that the differences between plants from meadows and pastures constitute the adaptive result of divergent selection between parcels of different land use. These findings of adaptation to altitude and land use matter for the present study, because adaptive evolution may have depleted heritable variation within grasslands. However, the higher allocation to reproduction of genotypes from pastures and the higher frequency of sexually reproducing plants at lower altitudes may suggest that heritable variation is higher among genotypes from pasture parcels than from meadow parcels, and among genotypes from parcels at lower altitudes. Higher heritable variation could also be maintained in grazed parcels than in mown parcels because of the spatially more heterogeneous selection by grazing animals. The relationship between land use and heritable genetic variation is of particular interest in the Alps, because land use is undergoing major changes there and many meadows are converted to pastures (Bätzing, 2003).

In Switzerland three different cultural traditions, the Germanic, Romanic, and Walser ones, are found in the Alps. With their specific farming practices these cultural traditions have contributed to the high diversity of the alpine landscapes (Bätzing, 1991). In the valleys of Romanic regions, more diverse types of land use are still found than in the valleys of the other cultural traditions (Chapter 1). If plants disperse between differently used parcels of grassland, the higher land use diversity in Romanic regions may suggest that heritable genetic variation of *P. alpina* is also higher among genotypes from grassland parcels from Romanic regions.

Heritable genetic variation may also be affected by soil conditions. Spatial or temporal heterogeneity of soil nutrients may cause heterogeneous selection, which in turn maintains heritable genetic variation (Stearns, 1992). Higher amplitudes of such heterogeneity is possible in grassland parcels with higher nutrient levels. Moreover, in grassland with higher nutrient availability the nutrient-indicator *P. alpina* (Landolt, 1977; Ellenberg et al., 1992) is

likely to be more abundant. Higher abundance is likely to increase heritability because it reduces the likelihood of genetic drift (Fischer & Matthies, 1998).

Heritable genetic variation may also be affected by the diversity of the surrounding community. Higher plant species richness may increase heritable variation in *P. alpina*, if it increases the diversity of available niches (Odat et al., 2004; Vellend & Geber, 2005). However, relationships between plant species diversity and quantitative genetic diversity have not been studied. Moreover, a more diverse community of parasites may impose more heterogeneous selection than a less diverse one, and may thus maintain higher heritable variation in plants. However, the relationships between the diversity of herbivores and pathogens and heritable variation in plants are not known.

We estimated broad-sense heritability of several vegetative and reproductive characters of *P. alpina* in a common garden experiment for 53 grassland parcels that represent different altitudes and land use around 12 villages along a 170 km east-west gradient in the Swiss Alps. To account for potential cultural differences we had selected four villages of each of the three main cultural traditions in the Swiss Alps, Romanic, Germanic, and Walser. The parcels were either mown or grazed and they were either additionally fertilized or unfertilized. Plant species diversity, the diversity of herbivory and fungal pathogen infection, and microsatellite diversity of *Poa alpina* were known for all parcels from previous studies.

We addressed the following questions: (1) Is there heritable genetic variation in vegetative and reproductive traits in *P. alpina*? Is such heritable genetic variation related to (2) topographical and soils parameters, (3) land use and cultural traditions, and (4) the diversity of surrounding vegetation, of herbivory, and fungal pathogens, and of microsatellite markers at plant origin?

Materials and methods

Study species

The Alpine Meadow Grass *Poa alpina* L. (Poaceae) is widespread in mountain regions in the Northern hemisphere (Conert, 1998). It occurs mainly on rich soils or alluvial plains and indicates high levels of nutrients and moisture. As a pioneer species, it also colonizes stony and rocky places of calcareous and silicate rocks, and exposed slopes and ridges. In the Swiss Alps, it occurs in natural sites up to 4200 m asl and in agriculturally used land between 550 and 2500 m asl (Conert, 1998). Due to its high fat and protein contents *P. alpina* is one of the most important fodder grasses in mountain meadows and pastures (Conert, 1998).

The perennial species occurs in seminiferous and pseudoviviparous forms. The latter ones reproduce by proliferation of the spikelet axis and production of bulbils instead of seeds. Some seminiferous plants produce seeds sexually, others via apomixis (Müntzing, 1933). Moreover, *Poa alpina* is a polyploid complex with common aneuploidy (Müntzing, 1980) and highly variable chromosome numbers (Steiner et al., 1997). In Switzerland chromosome numbers ranging from 2n = 22 to 46 have been reported (Duckert-Henriod & Favarger, 1987), whereas more than 60 chromosomes are known from Scotch plants (Müntzing, 1980).

Study sites

Study plants of *P. alpina* originated from 12 villages in the Swiss Alps, four of each of the three cultural traditions (named after the original settling tribes) Romanic, Germanic, and Walser (Chapter 1). The plants for this study were sampled in those 53 of a larger set of 216 grassland parcels in the Swiss Alps, where *P. alpina* occurred, and which represented unique combinations of village, altitudinal belt, and consistent land use. The 53 grassland parcels were situated at three altitudinal levels, 11 at the valley bottom (about 1000 m asl), 22 at intermediate altitudes (about 1500 m asl), and 20 at the alp level (about 2000 m asl). Parcels were fertilized (with liquid manure or dung) or unfertilized (i.e. never fertilized according to the farmer), and were currently mown or grazed. The unfertilized meadows were usually cut once a year whereas the fertilized meadows were cut between two and four times. Altogether, the parcels comprised 13 meadows, of which 6 were fertilized and 7 were not, and 40 pastures, of which 12 were fertilized and 28 were not.

Abiotic environment

From June to September 2002, we recorded the coordinates and altitude of the grassland parcels with a GPS, aspect as deviation from south in degrees, and slope in degrees to account for possible effects of topographic factors on the quantitative genetic diversity of *P. alpina*. To obtain concentrations of plant-available soil nutrients (potassium, calcium, magnesium, and phosphorous) we had soil samples from each parcel analyzed with the ammonium acetate EDTA method (AAEDTA 1:10) by Sol Conseil, Nyon, Switzerland. Additionally, we measured soil pH with a Hellige set (AVM Analyseverfahren, Freiburg, Germany).

Biodiversity measurements

As measure of landscape diversity around a village, we used the mean number of different land use types per village (Chapter 1). As measure of plant species diversity, we used the

mean number of plant species of two vegetation records per parcel, and Shannon's index of diversity and Evenness based on mean cover values of the two records (Chapter 1). As measure of the diversity of biological interactions per parcel, we used the number of different damage types by herbivory and by fungal pathogen infection per leaf and per parcel (Chapter 2). We measured molecular genetic diversity of *P. alpina* L. for six to eight plants per parcel at five polymorphic microsatellite loci (Maurer et al., 2005). We used the mean number of microsatellite alleles per plant and parcel and the mean allelic richness per parcel based on a rarefaction-sample size of six plants per parcel (Chapter 5).

Common garden experiment

For this study we considered two plants of each of six to eight genotypes of *P. alpina* of each of the 53 parcels grown in a common garden experiment at 1500 m asl at Davos, Switzerland, totaling 825 plants (Chapter 3). These plants had been collected from June to September 2002, and propagated and cut to standard size of 12 cm before planting into the experiment in May 2003. To one of the plants per genotype we applied a clipping-treatment to simulate land use, the other plant served as control.

Over two years, we measured several vegetative and reproductive characters. Of each plant, we harvested reproductive biomass, counted the number of reproductive shoots, and noted the mode of reproduction as seminiferous or pseudoviviparous. From the clipping-treatment plants, we obtained cumulative vegetative biomass sampled at four times during the two years (later on called clipped biomass). At the end of the experiment, we harvested aboveground vegetative biomass of each plant (later on called final vegetative biomass). From these data, we calculated total vegetative biomass by summing up clipped and final biomass, and total overall biomass by summing up total vegetative and reproductive biomasses. Moreover, we calculated the percentage of reproductive biomass relative to total biomass to obtain a measure for allocation to reproduction.

Statistical analysis

We calculated quantitative genetic diversity between genotypes within parcels. For each parcel we calculated broad-sense heritability of the measured quantitative characters separately (final vegetative biomass, total vegetative biomass, reproductive biomass, number of reproductive shoots, total overall biomass, and percentage of reproductive biomass). Moreover, we calculated the mean of the three heritabilites of reproductive characters, of the two heritabilities of vegetative characters, and of all six characters.

For each parcel, to calculate broad-sense heritability, we estimated variance components (VC) for variation among genotypes and for residual variation with expected mean squares (EMS) analyses of variance including the random factor genotype and the fixed factor treatment. The broad-sense heritability, H^2, is given by the quotient of the VC due to differences among genotypes (s^2_G) and the sum of this VC and the one due to residual variance (s^2_{Res}) as $H^2 = \frac{s^2_G}{s^2_G + s^2_{Res}}$ (Falconer & MacKay, 1996).

To analyze whether broad-sense heritabilities overall were significantly different from zero we used both possible tests. First, we tested whether variation among genotypes within grassland parcels was significant (Table 1A). To this end, we analyzed the reproductive and vegetative characters with analysis of variance (ANOVA) using a mixed model including the factors parcel, genotype, treatment, and parcel x treatment interaction. From this analysis, overall H^2 was obtained from the VC for genotypes and the VC residual. Second, we obtained mean and standard error of H^2 from all H^2 estimates across parcels. We used these data for a t-test of the test statistic mean/S.E. (Table 1B).

Further, we studied the relationship of topographical and soil characteristics of the parcels with broad-sense heritability estimates with pairwise Pearson product-moment correlations. Then, we analyzed effects of land use, altitude, and cultural traditions on broad-sense heritability with an analysis of variance (ANOVA) model with sequential sums of squares using a hierarchical mixed model including the factors culture, village, altitude, fertilization, current land use, and all two-way interactions. Effects of culture were tested against remaining variation among villages and effects of all other factors against variation due to remaining differences among parcels. To test potential relationships between heritabilities and abiotic parameters we included aspect, slope, geographic coordinates, and the soil nutrients K, Ca, Mg and P of the parcels as covariates into the model. As these did not change levels of significance, we used the model without covariates. Finally, we studied the relationships between heritability and the other parcel-measures of biodiversity with pairwise Pearson product-moment correlations.

Results

Broad-sense heritability in Poa alpina

Variation among genotypes within parcels was significant for all vegetative and reproductive characters (Table 1A). Corresponding broad-sense heritabilities H^2 were between 0.426 (percentage of reproductive biomass) and 0.647 (final vegetative biomass; Table 1A). In line

with the above, all means of the estimates of the single-parcel broad-sense heritability over all parcels were significantly different from zero and were between 0.363 (percentage of reproductive biomass) and 0.572 (final vegetative biomass; Table 1B).

The three heritability estimates for reproductive characters were non-significantly higher than the estimates of the two vegetative characters (t-test, df = 3; data of Table 1A: $t = 1.671$, $p > 0.19$; data of Table 1B: $t = 1.402$, $p > 0.26$).

Relationships between topographical and soil characteristics of grassland parcels and broad-sense heritability of Poa alpina

Geographic coordinates, aspect, and slope were not significantly correlated with the heritability estimates (Table 2). All correlations of soil nutrient concentrations and pH with heritability estimates were positive. Among these, soil concentrations of potassium, magnesium and phosphorous were significantly positively related with heritability estimates, especially of reproductive characters (Table 3). Whereas potassium concentration was significantly positively correlated to nearly all heritability estimates, concentrations of calcium and phosphorous were significantly positively correlated with only single estimates of broad-sense heritability (Ca: heritability of reproductive shoots, $N = 49$, $R = 0.314$, $p < 0.01$; P: heritability of total biomass, $N = 49$, $R = 0.308$, $p < 0.01$, Table 3). These correlations indicate that parcels with higher potassium soil concentrations are likely to host populations of *P. alpina* with higher broad-sense heritabilities.

Relationships between cultural traditions, land use, and altitude of grassland parcels and broad-sense heritability of Poa alpina

Broad-sense heritability of total vegetative biomass was higher for parcels from Romanic villages than for those from Germanic and Walser villages ($N = 53$, $F_{2, 9} = 5.1$, $p < 0.05$, Table 4, Fig. 1). Broad-sense heritabilities were higher for grazed parcels than for mown ones. This was especially pronounced for the heritability estimate of percentage of reproductive biomass ($N = 53$, $F_{1, 10} = 15.0$, $p < 0.01$), and also for overall mean heritability ($N = 53$, $F_{1, 10} = 6.5$, $p < 0.05$, Table 4, Fig. 2). Heritability estimates were independent of altitude and fertilization of grassland parcels.

Table 1 Broad-sense heritability H^2 of reproductive and vegetative characters measured in *Poa alpina* plants from 53 grassland parcels of different land use and altitude in the Swiss Alps. A) Broad-sense heritability estimated from within-parcel variance component among genotypes obtained by Analysis of Variance using data of all parcels (see methods). B) Broad-sense heritability estimated as mean across all single-parcel broad-sense heritabilities. Mean reproductive H^2 denotes mean of broad-sense heritability of three reproductive characters, mean vegetative H^2 denotes mean of broad-sense heritability of two vegetative characters, and mean overall H^2 denotes mean of all six broad-sense heritabilities.

	A		B			
	H^2	P (genotype)	Mean H^2	±SE	t=Mean H^2/SE	P (t-test)
Number of reproductive shoots	0.496	0.000	0.414	0.041	10.011	0.000
Reproductive biomass	0.513	0.000	0.425	0.041	10.342	0.000
% of reproductive biomass	0.647	0.000	0.572	0.046	12.485	0.000
Final vegetative biomass	0.426	0.000	0.363	0.035	10.269	0.000
Total vegetative biomass (clipped and final)	0.466	0.000	0.391	0.041	9.471	0.000
Total biomass (vegetative and reproductive)	0.446	0.000	0.394	0.039	10.065	0.000
Mean reproductive H^2			0.470	0.068	6.946	0.003
Mean vegetative H^2			0.377	0.014	26.805	0.001
Mean overall H^2			0.429	0.029	14.711	0.000

Table 2 Relationship between broad-sense heritabilities H^2 of reproductive and vegetative characters of *Poa alpina* and topographical parameters for 53 grassland parcels of different land use and altitude in the Swiss Alps. Pearson product moment correlation coefficients are given. X and Y coord denote geographical coordinates according to the Swiss Grid. Bold italic letters indicate marginally significant correlations ((*), $P < 0.1$).

	Altitude	X coord	Y coord	Slope	Aspect
Number of reproductive shoots	0.070	0.076	0.109	0.002	-0.048
Reproductive biomass	0.128	-0.075	-0.070	-0.044	-0.018
% of reproductive biomass	-0.121	-0.166	-0.010	-0.053	0.172
Final vegetative biomass	0.191	0.157	0.076	0.187	0.025
Total vegetative biomass (clipped and final)	0.130	0.093	-0.048	0.160	-0.108
Total biomass (vegetative and reproductive)	***0.242***	-0.119	-0.166	0.100	-0.069
Mean reproductive H^2	0.046	-0.070	0.007	-0.003	0.045
Mean vegetative H^2	0.163	0.127	0.010	0.178	-0.049
Mean overall H^2	0.164	-0.015	-0.030	0.114	-0.012

Table 3 Relationship between broad-sense heritabilities H^2 of reproductive and vegetative characters of *Poa alpina* and soil parameters for 53 grassland parcels of different land use and altitude in the Swiss Alps. Pearson product moment correlation coefficients are given. Bold letters indicate significant correlations (**, $P < 0.01$, *, $P < 0.05$), bold italics indicate marginally significant correlations ((*), $P < 0.1$).

	K	Mg	Ca	P	pH
Number of reproductive shoots	***0.243***	**0.333**	**0.314**	0.162	***0.264***
Reproductive biomass	**0.350**	0.202	0.137	0.178	0.126
% of reproductive biomass	**0.307**	0.227	0.083	0.095	0.196
Final vegetative biomass	0.209	***0.254***	0.140	0.170	0.029
Total vegetative biomass (clipped and final)	0.180	0.215	0.177	0.184	0.059
Total biomass (vegetative and reproductive)	**0.361**	0.183	0.149	**0.308**	0.075
Mean reproductive H^2	**0.341**	**0.295**	0.200	0.158	0.230
Mean vegetative H^2	0.200	***0.241***	0.165	0.184	0.047
Mean overall H^2	**0.371**	**0.322**	0.220	***0.241***	0.175

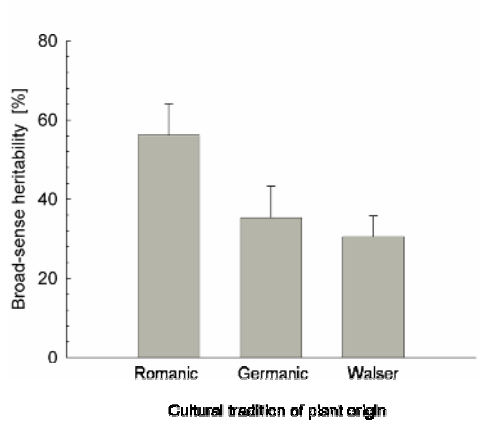

Figure 1 Relationship between cultural tradition and broad-sense heritability H^2 of total vegetative biomass of *Poa alpina* plants originating from 53 grassland parcels of different cultural traditions, land use and altitude in the Swiss Alps. Error bars denote 1 S.E.

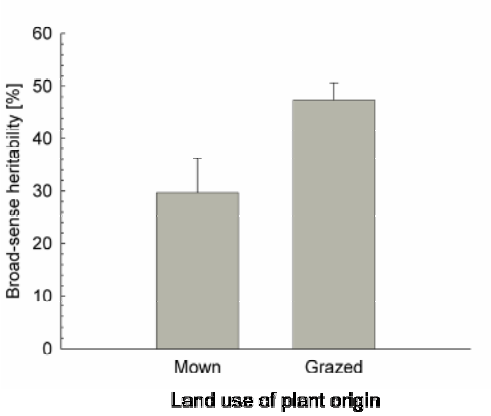

Figure 2 Relationship between land use and the mean overall broad-sense heritability H^2 of plants of *Poa alpina* originating from 53 grassland parcels of different cultural traditions, land use and altitude in the Swiss Alps. Error bars denote 1 S.E.

Relationships between community biodiversity and broad-sense heritability of Poa alpina

Broad-sense heritabilites were independent of plant species richness in the parcels of origin, and they were negatively related to the diversity of biological interactions within parcels (Table 5). Heritability H^2 of total vegetative biomass was negatively related to the diversity of herbivory and fungal pathogen infection. This was significant for the diversity of herbivory per grassland parcel, ($N = 49$, $R = -0.369$, $p < 0.01$) and marginally significant for the diversity of herbivory per leaf ($N = 49$, $R = -0.245$, $p = 0.09$) and for the diversity of fungal pathogen infection per leaf ($N = 49$, $R = -0.271$, $p = 0.06$, Table 5).

Relationship between microsatellite diversity and broad-sense heritability of Poa alpina

Heritability estimates of total overall biomass and of final vegetative biomass were marginally significantly positively related with mean allelic richness of microsatellite markers per parcel (Table 5), suggesting positive relationships between selectively neutral and quantitative genetic variation.

Table 4 Mean broad-sense heritabilities H^2 of different reproductive and vegetative characters of *Poa alpina* plants from 53 grassland parcels of different cultural tradition and land use in the Swiss Alps. Significant differences are indicated by bold numbers. SE denotes standard error. N denotes the number of parcels of plant origin.

		Mean H^2 ± SE							
		Reproductive characters				Vegetative characters			
	N parcels	Number of reproductive shoots	Reproductive biomass	% of reproductive biomass	Mean reproductive H^2	Final vegetative biomass	Total vegetative biomass (clipped and final)	Total biomass (vegetative and reproductive)	Mmean overall H^2
Romanic	15	0.405 ± 0.081	0.492 ± 0.059	**0.742 ± 0.058**	0.546 ± 0.056	**0.495 ± 0.066**	**0.562 ± 0.079**	0.448 ± 0.083	**0.524 ± 0.059**
Germanic	15	0.442 ± 0.075	0.429 ± 0.085	**0.651 ± 0.089**	0.507 ± 0.072	**0.322 ± 0.057**	**0.353 ± 0.080**	0.376 ± 0.077	**0.429 ± 0.050**
Walser	23	0.400 ± 0.065	0.376 ± 0.067	**0.408 ± 0.067**	0.401 ± 0.056	**0.304 ± 0.054**	**0.305 ± 0.053**	0.371 ± 0.054	**0.365 ± 0.041**
Mown	13	0.358 ± 0.094	0.287 ± 0.091	**0.297 ± 0.098**	**0.323 ± 0.088**	**0.227 ± 0.071**	0.273 ± 0.073	0.273 ± 0.063	**0.289 ± 0.059**
Grazed	40	0.43 ± 0.046	0.466 ± 0.044	**0.661 ± 0.044**	**0.519 ± 0.036**	**0.407 ± 0.039**	0.430 ± 0.048	0.434 ± 0.046	**0.471 ± 0.031**

Table 5 Relationship between broad-sense heritabilities H^2 of reproductive and vegetative characters of *Poa alpina* and landscape diversity of 12 villages and community diversity, and microsatellite diversity of *Poa alpina* for 53 grassland parcels of different land use and altitude in the Swiss Alps. Pearson product moment correlation coefficients are given. Bold letters indicate significant correlations (**, $P < 0.01$, *, $P < 0.05$), bold italics indicate marginally significant correlations ((*), $P < 0.1$).

	Number of land use types	Number of plant species	Diversity of herbivory per leaf	Diversity of herbivory per parcel	Diversity of fungal pathogens per leaf	Diversity of fungal pathogens per parcel	Mean number of alleles per plant	Mean allelic richness of six plants per parcel
N	12	49	49	49	49	49	53	53
Number of reproductive shoots	0.298	0.048	-0.107	-0.088	-0.038	0.046	-0.134	-0.103
Reproductive biomass	-0.021	-0.013	-0.156	-0.041	-0.060	0.070	-0.207	0.173
% of reproductive biomass	-0.137	-0.117	-0.079	-0.085	-0.025	-0.023	-0.09	0.108
Final vegetative biomass	0.176	0.039	-0.146	-0.168	***-0.269***	-0.130	0.017	***0.234***
Total vegetative biomass (clipped and final)	0.231	0.156	***-0.24)***	**-0.369**	***-0.271***	-0.053	-0.093	0.179
Total biomass (vegetative and reproductive)	-0.018	0.016	-0.149	-0.206	-0.072	0.072	-0.183	***0.269***
Mean reproductive H^2	0.004	-0.045	-0.169	-0.095	-0.068	0.012	-0.189	0.081
Mean vegetative H^2	0.149	0.106	-0.206	**-0.286**	***-0.27)***	-0.091	-0.044	0.212
Mean overall H^2	0.114	0.021	-0.239	-0.231	-0.185	-0.023	-0.183	0.204

Discussion

Broad-sense heritability

Compared with other species, the observed estimates of broad-sense heritability of vegetative and reproductive characters ranging from 0.363 to 0.572 (Table 1) appear intermediate to high. Significant broad-sense heritability of 0.198 was reported for fitness-relevant spatial spread in the clonal plant *Ranunculus reptans* (Fischer et al., 2004). In the grass *Eragrostis tef* a wide range of broad-sense heritabilities between 0.17 for shoot phytomass per plant and 0.74 for panicle length were reported (Assefa et al., 2001). The observed broad-sense heritabilities suggest that a high potential for further adaptation and evolution has been maintained in *P. alpina* even after hundreds of years of selection by agricultural land use.

The relatively high estimates of broad-sense heritability in *P. alpina* may possibly be related to the polyploidy and highly variable chromosome numbers of the species (Müntzing, 1980). For combinatory reasons, there are more possibilities for genetic polymorphisms among polyploid plants than among diploid plants. Moreover, if gene flow among plants of different ploidy levels is restricted (Meirmans et al., 2003), high genotypic variation and broad-sense heritability may be maintained. However, such relationships between quantitative genetic variation and within-species variation in polyploidy have not been explored.

Determinants of heritability

Topography and soil

Our results suggest that the variation in broad-sense heritability between grasslands with *P. alpina* is rarely affected by topography (Table 2). In particular, the higher frequency of sexual reproduction in plants from lower altitudes was not associated with higher broad-sense heritability in parcels at lower altitudes. In contrast, all relationships between broad-sense heritabilities and altitude were non-significantly positive. Possibly, a positive effect of higher frequency of sexual reproducing plants at lower altitudes on broad-sense heritability was superimposed by a negative one of increased fragmentation of grasslands at lower altitudes. The latter was brought forward to explain the higher molecular genetic variation within populations of *Primula farinosa* from higher altitudes (Reisch et al., 2005).

Heritable genetic variation was higher for *P. alpina* from parcels with higher soil nutrient concentrations, especially potassium (Table 3). This may be due to higher spatial or temporal variation in nutrient concentrations in such parcels, which may have led to more heterogeneous selection and thus may have maintained higher heritable genetic variation (Stearns, 1992). Nutrient-rich mountain grassland and hence typical sites for *P. alpina* are

usually adequately supplied with potassium according to the nearly closed biological cycle of potassium due to complete recirculation by dung (Bohner & Sobotik, 2000). Accordingly, the higher abundance of the nutrient indicator *P. alpina* in more nutrient rich parcels may have reduced negative effects of genetic drift on heritability (Willi et al., 2005), which may explain the positive relationship between soil potassium concentration and heritability of *P. alpina*.

Current land use and cultural traditions

The broad-sense heritability estimates of several reproductive and vegetative characters in *P. alpina* were on average twice as high for grazed than for mown parcels. These results are even more pronounced than the ones of our microsatellite study of *P. alpina* where the mean number of alleles was also higher in grazed parcels than in mown ones (Chapter 5). The higher heritable genetic variation in grazed parcels is in line with the hypothesis that increased allocation to reproduction of plants in grazed parcels increases establishment of new genotypes, and thus genetic variation. Moreover, higher allocation to reproduction may also increase gene flow. Because for topographical reasons grazed parcels are somewhat more likely to be situated next to other grazed parcels than to mown parcels, this can also contribute to higher genetic variation within grazed parcels. Higher gene flow among grazed parcels with *P. alpina* was indeed indicated by the lower microsatellite differentiation between grazed than between mown parcels with *P. alpina* (Chapter 5). An alternative explanation of reduced heritable genetic variation in mown sites could be more spatially homogeneous selection by mowing than by grazing animals. Accordingly, intensive mowing reduced molecular genetic diversity in *Festuca pratensis* compared to natural populations (Kölliker et al., 1998).

The observed higher heritable genetic variation of *P. alpina* for grassland parcels from Romanic regions indicates persisting effects of old cultural traditions on current biodiversity, in this case intraspecific diversity. Most likely this is due to the higher land use diversity especially in the valleys of Romanic villages (Chapter 1). We had shown earlier that *P. alpina* is genetically differentiated between parcels of different land use (Chapter 3). Therefore, gene flow between parcels with *P. alpina* of different land use is likely to increase heritable genetic variation within parcels. The occurrence of such gene flow is the more likely the more different types of land use occur around a village. Most likely, the higher heritable genetic variation for parcels in Romanic villages is at least partly due to historical rather than to current gene flow, as it was more pronounced in the past, and will decrease in the near future, as far as ongoing land use changes are reducing land use differences between villages of different cultural traditions (Bätzing, 1991).

Land use turned out to be the most important driver of heritable genetic variation in *P. alpina*. This implies that the ongoing rapid land use change in the Alps, where many meadows are converted to pastures (Bätzing, 2003), will lead to increased heritable genetic variation within grassland parcels, at least of *P. alpina*. However, because higher land use diversity also increases quantitative genetic variation between sites, the conservation of quantitative genetic variation requires the conservation of different types of land use, and cannot be achieved by solely maintaining grazed parcels.

Other levels of biodiversity

Our results revealed weak relationships between heritable genetic variation and plant community diversity (Table 5). Thus, our study provides little support for the hypothesis, that plant community diversity could be positively related to heritable genetic variation, because more diverse niches are available in communities that are more diverse. Plant species diversity was weakly positively related with molecular genetic diversity in the forest herb *Trillium grandiflorum* (Vellend, 2004). In our study heritable genetic variation was marginally significantly positively related to molecular genetic diversity (Table 5). If this holds true also in *T. grandiflorum*, it may suggest that plant species diversity is also positively correlated with heritable genetic variation in *T. grandiflorum*. However, positive relationships between plant species diversity and molecular genetic variation were absent in *Ranunculus acris* (Odat et al., 2004) and present, but explained by confounding habitat characteristics, in *Plantago lanceolata* (N. Odat, Helwig, Jeschke, and M. Fischer, unpublished data). We suggest to study the relationship between plant species diversity and genetic diversity for more plant species and not only for molecular, but also for heritable, genetic variation.

The generally non-significant and negative relationships between the diversity of herbivores and pathogens and heritable variation in *P. alpina* (Table 5) appear to not support the hypothesis that a more diverse community of parasites may impose more heterogeneous selection than a less diverse one, and may thus maintain higher heritable variation in plants. However, such negative relationships were in general very weak, and the relationship between the diversity of fungal parasitism per parcel and heritable variation of half of the measured traits in *P. alpina* was non-significantly positive (Table 5). Therefore, the hypothesis should not be dismissed completely. We suggest clarifying these relationships more comprehensively by studying heritable genetic variation of not just one, but several plant species in relation to the diversity of the plant community, and the diversity of plant parasitism. For the latter, it will be interesting to compare the relationships between heritable genetic variation of plants

and the diversity of parasites for two cases, first for all parasites in a grassland, and second, only for the parasites actually affecting the target species.

Conclusions

The observed broad-sense heritabilities suggest that a high potential for further adaptation and evolution has been maintained in *P. alpina* even after hundreds of years of selection by agricultural land use. Among the potential drivers of heritable genetic variation in *P. alpina*, land use was most important with heritable genetic variation halved in meadows compared with pastures. Because unfertilized meadows harbor the most species-rich plant communities (Chapter 1), and because the higher land use diversity in Romanic villages was associated with higher species diversity and higher heritable genetic variation, we suggest maintaining diverse land use regimes to conserve biodiversity not only at the landscape and plant species level, but also at the level of heritable variation within species, at least of the widespread important fodder grass *Poa alpina*.

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Summary

The unique cultural landscape of the Alps has been shaped by hundreds of years of anthropogenic land use. Different land use types created a diverse mosaic of meadows and pastures that replaced the formerly dominating forests. Therefore, grassland is an important element contributing to the biodiversity of alpine regions. Biodiversity comprises several levels of biological integration including the diversity of landscape and habitats, the diversity of species, the diversity of biological interactions (such as between plants and herbivores, plants and pathogens, and plants and pollinators), and the genetic diversity within a species. High biodiversity is of aesthetical and ethical value, and of ecological and economic value, as it stabilizes ecosystems and increases the stress tolerance of communities. Accordingly, the importance of biodiversity has been acknowledged in the Convention on Biological Diversity of the United Nations in 1992.

Since then, and stimulated by the convention, biodiversity has become a research focus. Scientific studies mainly covered single aspects of biodiversity by focussing on species richness, on genetic diversity, or on the relationship between experimentally manipulated plant species diversity and ecosystem functioning. However, so far comprehensive studies addressing several levels of biodiversity are lacking.

Ongoing socio-economically motivated changes in land use are especially apparent in mountain regions and are about to negatively affect the biodiversity of grassland there. Therefore, we selected 216 grassland parcels of different land use and altitude in 12 villages in the Swiss Alps to examine anthropogenic and natural effects on several levels of biodiversity. To account for regional differences due to settlement history, we included the three cultural traditions Romanic, Germanic/Alemannic, and Walser into our design, as they are likely to have influenced land use in the specific regions. In a hierarchical parcel-based design, we studied the effects of cultural traditions, altitude, and land use on different levels of biodiversity.

Chapter 1 examines the level of plant species diversity and the diversity of land use types. We analyzed the presence of different land use types occurring in the study villages. Moreover, we performed two vegetation records each in 216 grassland parcels of different cultural traditions, altitude, and land use to estimate plant species richness. In Romanic villages, more different parcel types tended to occur in the valley than in Germanic and Walser ones, suggesting that socio-economic differences among cultural traditions still play a role in shaping landscape diversity. At the village level, higher man-made landscape diversity,

i.e. a higher number of land use types per village, was associated with higher plant species richness. We detected changes in land use exclusively from mowing to grazing or from managed to abandoned grasslands, both resulting in a reduction of workload for farmers. The highest plant species richness per parcel recorded at intermediate altitudes was due to overlapping species pools of lower and higher altitudes. Fertilization and abandonment reduced plant species richness. Grazing slightly reduced species richness compared to mowing in unfertilized parcels, while in fertilized parcels grazing had a positive influence. Overall, we found the highest species diversity in mown unfertilized subalpine grasslands. We conclude that observed changes in land use reduce plant species richness both within grassland parcels and at the landscape level. As a valuable alternative to abandonment, we suggest moderate grazing of former meadows. Clearly, financial incentives are needed to not only maintain those types of land use that conserve high plant species diversity within grasslands, but in addition to promote a high diversity of land use types in the landscape.

In **Chapter 2** we examined the extent and diversity of plant-herbivore and plant-pathogen interactions in mountain grassland by estimating the amount of leaf damage by, and the number of types of, herbivory and fungal pathogen infection. We sampled more than 12'000 leaves of plants of the three functional groups legumes, graminoids and other forbs in 215 of our study parcels. We were able to demonstrate the omnipresence of herbivores and fungal pathogens in mountain grassland indicating that plant-herbivore and plant-pathogen interactions contribute largely to community diversity. At the same time, leaf damage by herbivores and fungal pathogens was very moderate, even in the case of legumes and at lower altitudes where leaf damage was highest. Therefore, conserving the diversity of plant-herbivore and plant-pathogen interactions will not be at the expense of dramatic plant biomass losses. In parcels with higher number of plant species the diversity of herbivory types was lower at the leaf level, but tended to be higher at the parcel level. We found low altitudes and traditional mowing to promote plant-herbivore interactions, while fertilization negatively affected fungal pathogens. Therefore, current land use changes in the Alps are likely to decrease the diversity of plant-herbivore, and as far as they involve fertilization, of plant-pathogen interactions. As our results neither indicate a conflict between conservation goals for different taxa, nor a conflict between conservation and agricultural goals, we suggest maintaining high biodiversity of mountain grassland by diverse low-intensity land use.

In **Chapter 3**, first we analyzed the occurrence of the Alpine Meadow Grass *Poa alpina* in the field. Among the 216 grassland parcels, we found it most frequently occurring in fertilized and grazed sites and at higher altitudes. Second, we report on a common garden experiment examining the intraspecific diversity of *P. alpina,* one of the most important fodder grasses in the Swiss Alps. We grew two plants of each of 615 genotypes originating from 57 grassland parcels and 21 natural sites and harvested reproductive and vegetative biomass over two years. One plant per genotype was clipped to simulate land use, and one served as control. Plants from lower altitudes reproduced largely via seeds, whereas those from higher altitudes rather reproduced vegetatively via bulbils, in line with the hypothesis that vegetative reproduction is adaptive at higher altitudes, where conditions are harsher for seedling recruitment. In plants from grazed sites, we observed higher allocation to reproduction than in plants from mown sites, in line with the hypothesis that recruitment is of higher adaptive value in grazed sites, which offer more suitable microsites for recruitment than the more homogeneous mown grasslands do. Intermediate allocation to reproduction of plants from unused natural sites suggests that *P. alpina* has undergone divergent selection in response to mowing and grazing. We conclude that intraspecific phenotypic differences between plants originating from parcels of different altitude and land use have a genetic component, which is shaped by adaptation to both natural and anthropogenic influences.

In **Chapter 4** we describe the development of microsatellite markers in *Poa alpina*. We characterized five polymorphic microsatellite loci. Moreover, using DNA of 25 plants of *Poa alpina* and chromosome counts in root tips of these plants we report that more microsatellite alleles were found for plants with higher chromosome numbers. Moreover, using DNA of > 400 plants from 54 parcels of land we show that the genetic distance between parcels of land increased slightly with increasing geographic distance. These first tests show that the developed microsatellite markers are indeed suitable for the study of molecular genetic variation.

Chapter 5 examines the molecular intraspecific diversity of *Poa alpina.* After DNA extraction we used the microsatellite markers described in Chapter 4 to analyze the molecular intraspecific diversity of 569 *P. alpina* plants originating from 20 natural sites and 54 grassland parcels of different cultural tradition, land use, and altitude. As measure for genetic diversity we calculated the mean number of alleles per plant and the mean allelic richness based on six plants per population. 1.1 % of the molecular variation in the presence and

absence of alleles was between natural and agriculturally used parcels while there was no difference in within-parcel genetic diversity. 25.1% of the molecular variation was among parcels, and 74.9% within. Of the 25.1 % variation among parcels, 6.8 % were among villages, 4.2 % between parcels with seed-producing and parcels with bulbil-producing plants, and 1.3 % between mown and grazed parcels. Within villages, more different land use types enhanced genetic diversity. Within populations, grazing enhanced genetic diversity compared to mowing. At higher altitudes allelic richness per population increased with altitude. These results demonstrate that agricultural land use has left its marks in the genome of *P. alpina.* This confirms the experimental results of Chapter 3, that land use affects biodiversity not only at the species level (Chapter 1) and the level of biological interactions (Chapter 2), but also within a species common in the Swiss Alps. This implies that ongoing socio-economic changes will not only act at the landscape or community level but also change the diversity within single species. As the genomes of plants growing in meadows and pastures have differentiated, we conclude that the support of not only one single type of land use but rather a high diversity of land use types will promote the highest within-species diversity.

In **Chapter 6** we examined the size of heritable genetic variation in the Alpine Meadow Grass *Poa alpina* by studying the broad-sense heritability of several reproductive and vegetative characters measured in the common garden experiment reported in Chapter 3. For this study, we included 825 *P. alpina* plants originating from 53 grassland parcels of different cultural tradition, land use, and altitude. We asked for the main abiotic, natural, and anthropogenic determinants of broad-sense heritability, and its relationship to different levels of biodiversity. In the experiment, significant variation among genotypes within parcels of origin indicated significant broad-sense heritability within parcels. Estimates of broad-sense heritability were moderate to high. Broad-sense heritabilities were larger in plants from parcels with higher concentration of potassium, possibly because populations of *P. alpina* might be larger there. This indicates that soil nutrient levels play a role for the genetic variation in *P. alpina*. In plants originating from grazed parcels, estimates of broad-sense heritability were more than two times higher than in mown ones. The tendency of higher broad-sense heritability among *P. alpina* plants from parcels from Romanic villages than from those from Germanic and Walser villages indicate regional differences brought about by old cultural traditions. It is very well possible, that this finding was due to the higher numbers of different land use types in the valley of Romanic villages reported in Chapter 1. While broad-sense heritability was lower in parcels with lower diversity of herbivory, it was not correlated with the diversity of

plant species and fungal pathogens. The results of this chapter show that the hundreds of years of selection by land use turned out to be the most important determinant of quantitative genetic variation in *P. alpina*. The severe reduction of genetic variation by mowing, which on the other hand had promoted higher plant species diversity in unfertilized sites (Chapter1), leads us to suggest promoting diverse land use regimes to conserve not only landscape diversity and plant species diversity (Chapter1), but also the intraspecific diversity of *P. alpina*.

Conclusion

Our comprehensive study across 12 villages along a 170 km east-west gradient in the Swiss Alps enabled us to show that human land use affects biodiversity at all levels. This indicates that biodiversity has largely been shaped by hundreds of years of human activity. Moreover, we showed that cultural traditions still affect man-made landscape diversity. Still persisting socio-economic differences among villages of different cultural traditions are likely to be responsible for this cultural influence.

The observed conversions in land use in the direction of reduced labor for farmers will reduce land use diversity and at the same time, the number of high-biodiversity parcels used at low intensity will decrease. Thus, biodiversity will decrease both between and within parcels of land. Therefore, the ongoing changes in agriculture need to be halted in order to not risk high losses of alpine biodiversity. This makes extended financial incentives for biodiversity-promoting land use necessary. Such financial incentives should not only promote high biodiversity within parcels of land, but also between parcels. Therefore, these incentives should not only address the land use of single parcels, but also the diversity of land use of different parcels of a farm or a village.

Plant species richness was highest in unfertilized mown parcels. Because mowing is especially laborious at steep slopes at high altitudes, financial incentives are especially important for the conservation of the remnants of flower rich meadows at high altitudes. Therefore, such subsidies are even justified when parcels that have been left abandoned for some years are started to be used again, as it is already successfully done in the canton of Grisons.

In contrast to widespread reservations against grazing, we showed that particularly low-intensity grazing has several positive aspects, concerning landscape diversity, plant species diversity, intraspecific diversity, and the promotion of biological interactions. Moreover, from a biodiversity point-of-view grazing clearly is preferable to abandonment of

grassland use. All in all, in concert with mown sites, grazed sites contribute significantly to the biodiversity of grassland in the Alps.

In summary, for biodiversity at all levels of biological integration, it is highly important to promote a highly diverse landscape. Therefore, financial incentives should aim at high land use diversity, which is likely to be most successfully promoted at the village level. Increasingly monotonic agricultural land use or even abandonment of whole regions will drastically reduce biodiversity, and at the same time, it will reduce landscape attractiveness for tourists and endanger the remnants of cultural heritage in the Swiss Alps. The conservation of all levels of biodiversity, and of their cultural, aesthetical, ecological, and economic values requires the persistence of the diverse landscapes of the Alps.

Zusammenfassung

Biodiversität umfasst mehrere Ebenen der biologischen Vielfalt: die Vielfalt von Lebensräumen und Landschaften, die Artenvielfalt, die Vielfalt von biologischen Wechselwirkungen (z.B. zwischen Pflanzen und Herbivoren, Pflanzen und Pathogenen, sowie Pflanzen und ihre Bestäuber) und die genetische Vielfalt innerhalb einer Art. Eine hohe Biodiversität hat bedeutenden ästhetischen und ethischen, sowie ökonomischen und ökologischen Wert, da sie Ökosysteme stabilisiert und die Stresstoleranz von Arten erhöht. Dieser hohen Relevanz von Biodiversität wurde in der Biodiversitätskonvention der Vereinten Nationen 1992 Rechnung getragen.

Seit den frühen 90er Jahren und durch die Biodiversitätskonvention angeregt rückte Biodiversität verstärkt ins Interesse der Forschungsgemeinde. Wissenschaftliche Studien befassten sich vornehmlich mit einzelnen Aspekten von Biodiversität, indem sie vor allem Artenvielfalt in bestimmten Habitaten, genetische Diversität einzelner Arten, oder den Zusammenhang von experimentell manipulierter Artenvielfalt und Ökosystemfunktionen untersuchten. Hingegen fehlten bisher Studien, die sich übergreifend mehreren Ebenen der Biodiversität widmeten.

Die einzigartige Kulturlandschaft der Alpen ist im Laufe von Jahrhunderten durch menschliche Nutzung gewachsen. Durch unterschiedliche Landnutzungsarten entstand ein vielfältiges Mosaik aus Wiesen und Weiden, das die ehemals dichten Wälder ersetzte. Dadurch leistet Grasland einen wichtigen Beitrag zur hohen Biodiversität in den Alpen. Vor allem das Berggebiet ist zunehmend von sozioökonomischen Veränderungen in der Landwirtschaft betroffen, so dass negative Auswirkungen auf die Biodiversität von Grasland zu erwarten sind.

Für unsere Studie wählten wir 216 landwirtschaftliche Parzellen von unterschiedlicher Nutzung und Höhenlage in 12 Gemeinden der Schweizer Alpen aus, um menschliche und natürliche Einflüsse auf die verschiedenen Ebenen der Biodiversität von Grasland zu untersuchen. Um auch regionalen und siedlungshistorischen Unterschieden gerecht zu werden, integrierten wir die drei sogenannten kulturellen Traditionen romanisch, germanisch-alemannisch und Walser in unser Studiendesign, da diese die Art der Landwirtschaft in den jeweiligen Regionen möglicherweise mitbeeinflusst haben. Mithilfe eines hierarchischen parzellenbasierten Versuchsdesigns untersuchten wir die Einflüsse von kulturellen Traditionen, Höhe und Landnutzung auf die unterschiedlichen Ebenen der Biodiversität.

Kapitel 1 ist der Vielfalt von Pflanzenarten und Landnutzungstypen gewidmet. Wir untersuchten das Vorkommen verschiedener Landnutzungstypen in den jeweiligen Gemeinden. Ausserdem führten wir zwei Vegetationsaufnahmen in jeder der 216 ausgewählten Parzellen unterschiedlicher Kultur, Höhe und Landnutzung durch, um ein Mass für die Artenvielfalt zu bekommen. In romanischen Gemeinden gab es tendenziell mehr verschiedene Landnutzungstypen auf der Talstufe als in germanisch-alemannischen und Walser Gemeinden, was darauf hinweist, dass sozioökonomische Unterschiede zwischen den kulturellen Traditionen noch heute vorhanden sind und die Landschaftsdiversität beeinflussen. Auf der Talstufe ging eine hohe Anzahl an verschiedenen Landnutzungstypen mit einer hohen Pflanzenartenzahl einher. Veränderungen in der Landnutzung verliefen entweder von Mahd zu Beweidung, oder allgemein von Nutzung zu Verbrachung, d.h. immer zur Arbeitsverminderung für die Landwirte. Die untersuchte hohe Artenzahl in der mittleren Stufe spiegelt eine Überlappung der Artenpools aus den Hochlagen und aus tiefen Lagen wider. Düngung und Verbrachung führten zu einer Reduktion der Artenzahl. In ungedüngten Flächen reduzierte Beweidung die Artenvielfalt geringfügig im Vergleich zu gemähten Flächen, während in gedüngten Flächen die Artenzahl auf Weiden höher war als auf Wiesen. Die höchste Artenzahl wiesen ungedüngte gemähte Flächen der subalpinen Stufe auf. Daraus folgern wir, dass die beobachteten Nutzungsänderungen die Artenvielfalt sowohl innerhalb der Parzellen als auch auf Landschaftsebene reduzieren. Alternativ sollte versucht werden, früher gemähte Flächen heute zu beweiden anstatt sie brach fallen zu lassen. Dazu sind eindeutige finanzielle Anreize für die Landwirte notwendig, um nicht nur eine Vielzahl an Landnutzungstypen zu fördern, die eine hohe Pflanzenartenvielfalt erbringen, sondern die auch zur Vielfalt der Landschaft beitragen.

In **Kapitel 2** untersuchten wir das Ausmass und die Vielfalt von Wechselwirkungen von Pflanzen und Herbivoren sowie von Pflanzen und Pathogenen in Bergwiesen. Dazu sammelten wir mehr als 12'000 in 215 unserer Unterhuschungsparzellen gesammelten Blättern die betroffene Fläche und zählten die verschiedenen Typen Herbivoren und Pathogenen. Die Blätter stammten von den drei funktionellen Gruppen Leguminosen, Grasartige und andere Kräuter. Wir konnten nachweisen, dass Herbivoren und Pathogene auf allen Bergwiesen vorkommen und somit entscheidend zur Vielfalt von Lebensgemeinschaften beitragen. Gleichzeitig blieb das Ausmass des Schadens durch Herbivoren und Pathogene gering, auch bei Leguminosen und in niedriger Höhenlage, wo im Vergleich das Ausmass des Schadens am höchsten war. Somit ist durch den Schutz der Pflanzen-Herbivoren- und

Pflanzen-Pathogen-Interaktionen nicht mit schwerwiegenden Biomasseverlusten zu rechnen. In Parzellen mit vielen Pflanzenarten war die Vielfalt von Herbivorietypen auf Blattebene geringer, auf Parzellenebene tendenziell aber höher. Niedrige Höhenlage und Mahd förderten Pflanzen-Herbivoren-Interaktionen, während Düngung Pflanzen-Pathogen-Interaktionen negativ beeinflusste. Aktuelle Landnutzungsänderungen in den Alpen scheinen demnach die Vielfalt an Pflanzen-Herbivoren-Interaktionen zu verringern, und sobald verstärktes Düngung involviert ist, auch die Pflanzen-Pathogen-Interaktionen. Da unsere Ergebnisse aber weder einen Konflikt beim Schutz verschiedener Taxa noch einen Konflikt zwischen Naturschutz und Landwirtschaft offenlegen, kommen wir zum Schluss, dass eine diverse extensive Landnutzung die Biodiversität von Bergwiesen fördert.

Im **3. Kapitel** untersuchten wir zunächst das Vorkommen des Alpenrispengrases *Poa alpina* in unserer Untersuchungsregion. Unter den 216 Parzellen kam es bevorzugt in gedüngten und beweideten Flächen vor, vor allem in höheren Lagen. Ausserdem berichten wir von einem sogenannten „Common garden experiment“, mit dem wir die innerartliche Diversität von *Poa alpina*, einem der am weitesten verbreiteten Futtergräser in den Alpen, untersuchten. Wir pflanzten je zwei Pflanzen von 615 Genotypen, die aus 57 landwirtschaftlich genutzten Parzellen und 21 natürlichen Standorten stammten, und ernteten reproduktive und vegetative Biomasse über zwei Jahre. Eine Pflanze eines Genotyps wurde geschnitten, um Landnutzung zu simulieren, die andere diente als Kontrolle. Pflanzen, die aus tieferen Lagen stammten, reproduzierten mehrheitlich durch Samen, während diejenigen aus höheren Lagen sich hauptsächlich vegetativ mittels Bulbillen fortpflanzten. Dies entsprach unserer Hypothese, dass sich Pflanzen durch vegetative Fortpflanzung an die erschwerten Keimungsbedingungen in höheren Lagen anpassen. In Pflanzen aus Weiden beobachteten wir eine erhöhte Allokation in reproduktive Biomasse im Vergleich zu Pflanzen aus Wiesen. Dies bestätigte unsere Hypothese, dass sich Pflanzen eher an heterogene Weiden mit ihren vielfältigen für die Keimung günstigen Kleinstandorten anpassen als an homogenere Wiesen. Die mittlere Allokation in Reproduktion von Pflanzen genutzter Standorte im Vergleich zu Pflanzen von ungenutzten natürlichen Standorten weist daraufhin, dass *Poa alpina* durch Mahd und Beweidung unterschiedlich selektioniert wurde. Wir schliessen daraus, dass innerartliche phänotypische Unterschiede zwischen den Pflanzen aus Flächen unterschiedlicher Höhe und Landnutzung auch genetisch bedingt sind, indem sie durch Anpassung an natürliche und anthropogene Faktoren geformt werden.

In **Kapitel 4** beschreiben wir die Entwicklung von Mikrosatellitenmarkern in *Poa alpina.* Hierbei konnten wir fünf polymorphe Loci für Mikrosatelliten ausweisen. Mithilfe von DNA aus 25 Pflanzen, in denen wir in Wurzelspitzen Chromosomen auszählten, zeigte sich, dass Pflanzen mit mehr Mikrosatellitenallelen auch mehr Chromosomen aufwiesen. Des Weiteren konnten wir mit DNA aus mehr als 400 Pflanzen von 54 landwirtschaftlich genutzten Parzellen nachweisen, dass die genetische Distanz zwischen den Parzellen mit zunehmender geographischer Distanz leicht zunahm. Diese ersten Tests bestätigten, dass sich die entwickelten Mikrosatellitenmarker eignen, um die genetische Vielfalt auf molekularer Ebene zu analysieren.

Kapitel 5 untersucht die molekulare innerartliche Diversität von *Poa alpina.* Nach der Extraktion von DNA verwendeten wir die in Kapitel 4 charakterisierten Mikrosatelliten, um die molekulare innerartliche Diversität von 569 Pflanzen aus 20 natürlichen Standorten und 54 landwirtschaftlich genutzten Parzellen zu analysieren. Als Mass für die genetische Diversität berechneten wir die mittlere Anzahl Allele pro Pflanze und die mittlere „Allelic richness“, die auf sechs Pflanzen pro Population basiert. 1.1% der molekularen Variation im Vorhandensein oder Nichtvorhandensein von Allelen fanden wir zwischen natürlichen und genutzten Flächen. Wir fanden keine Unterschiede im Genom von Pflanzen innerhalb derselben Parzelle. 25.1% der molekularen Variation war zwischen den Parzellen und 74.9% innerhalb. Von den 25.1% Variation zwischen den Parzellen resultierten 6.8% zwischen Gemeinden, 4.2% zwischen Parzellen mit samenproduzierenden und Parzellen mit bulbillen produzierenden Pflanzen, sowie 1.3% zwischen gemähten und beweideteten Parzellen. Innerhalb der Gemeinden förderte Beweidung die genetische Diversität im Vergleich zu Mahd. Innerhalb von Populationen waren Pflanzen aus Weiden genetisch diverser als solche aus Wiesen. Ausserdem nahm mit steigender Höhe auch die „Allelic richness“ zu. Diese Ergebnisse zeigen, dass ein durch die Landnutzung bedingter Selektionsdruck seine Spuren im Genom von *Poa alpina* hinterlassen hat, was die experimentellen Ergebnisse aus Kapitel 3 bestätigt: Landnutzung beeinflusst die Biodiversität nicht nur in der Vielfalt der Arten (Kapitel 1) und auf dem Level der biologischen Interaktionen (Kapitel 2), sondern auch innerhalb einer in den Schweizer Alpen weit verbreiteten Art. Dies legt nahe, dass sich fortdauernde sozioökonomische Veränderungen in der Landwirtschaft nicht nur auf Landschaftsebene oder auf die Ebene von Lebensgemeinschaften auswirken, sondern auch die genetische Vielfalt innerhalb einzelner Arten verändern. Die unterschiedliche Entwicklung des Genoms von Pflanzen aus Wiesen und Weiden zeigt, dass nicht nur *eine* bestimmte Art

von Landnutzung eine hohe innerartliche Diversität fördert, sondern eine hohe Vielfalt an Landnutzungstypen anzustreben ist.

Im **6. Kapitel** untersuchten wir den erblichen Anteil der genetischen Variation im Alpenrispengras *Poa alpina,* indem wir die Erblichkeit im weiteren Sinne (sog. „broad-sense heritability“) verschiedener reproduktiver und vegetativer Merkmale, die in dem im 3. Kapitel beschriebenen „Common garden experiment“ gemessen wurden, berechneten. Dazu verwendeten wir 841 Pflanzen aus 54 landwirtschaftlich genutzten Parzellen verschiedener kultureller Traditionen, Landnutzung und Höhe. Wir stellten die Frage nach abiotischen, natürlichen und anthropogenen Faktoren, die die Erblichkeit vor allem beeinflussen, sowie die Beziehung zwischen Erblichkeit und den anderen Ebenen der Biodiversität. Im Experiment festgestellte signifikante Variation zwischen den Genotypen innerhalb der Herkunftsparzellen deutete signifikante Erblichkeit innerhalb der Parzellen an. Die Werte für Erblichkeit waren mittelmäßig bis hoch. Die Erblichkeit war höher in Pflanzen von Parzellen mit höheren Kalziumgehalten im Boden, möglicherweise weil dort auch größere *Poa* Populationen wachsen. Dies deutet darauf hin, dass Konzentrationen von Nährstoffen im Boden eine Rolle für die genetische Variation in *P. alpina* spielen. In Pflanzen aus Weiden war die Erblichkeit mehr als zweimal so hoch wie in Pflanzen aus Wiesen. Eine tendenziell höhere Erblichkeit in Pflanzen aus romanischen als germanisch-alemannischen und Walser Gemeinden weist auf regionale Unterschiede aufgrund kultureller Traditionen hin. Möglicherweise besteht ein Bezug zur Vielfalt an Nutzungstypen im Tal romanischer Gemeinden (Kapitel 1). Während die Erblichkeit in Parzellen mit weniger Herbivorietypen ebenfalls geringer war, gab es keine Korrelation mit der Vielfalt von Pflanzenarten und Pathogenen. Die Ergebnisse dieses Kapitels zeigen, dass der seit Jahrhunderten andauernde Selektionsdruck durch Landnutzung der wichtigste Faktor ist, der die genetische Variation in *P. alpina* bestimmt. Die gravierende Homogenisierung der genetischen Variation durch Mahd, die umgekehrt die Pflanzenartenvielfalt in ungedüngten Flächen fördert (Kapitel 1), lässt uns auch hier zum Schluss kommen, dass eine diverse Landnutzung nicht nur die Vielflat der Landschaft und der Pflanzenarten (Kapitel 1), sondern auch die innerartliche Diversität von *P. alpina* fördert.

Schlussfolgerung

Mit dieser umfassenden Studie in 12 Gemeinden entlang eines 170 km Ost-West-Gradienten konnten wir belegen, dass Landnutzung die Biodiversität auf allen Ebenen beeinflusst. Dies bestätigt, dass die Biodiversität in den Schweizer Alpen ein Produkt der Jahrhunderte langen

menschlichen Aktivitäten ist. Wir konnten nachweisen, dass kulturelle Traditionen beeinflussen immer noch die vom Menschen gestaltete Vielfalt der Landschaft beeinflussen. Noch immer existierende sozioökonomische Unterschiede zwischen den Dörfern unterschiedlicher kultureller Traditionen sind möglicherweise verantwortlich für diesen Einfluß der kulturellen Traditionen.

Die beobachteten Nutzungsänderungen in Richtung der Arbeitsverminderung für die Landwirte werden die Vielfalt der Landnutzungstypen reduzieren und gleichzeitig zu einem Rückgang von extensiv genutzten Parzellen hoher Biodiversität führen. Dadurch wird die Biodiversität sowohl zwischen als auch innerhalb der Parzellen zurückgehen. Um gravierende Verluste der Biodiversität in den Alpen zu verhindern, müssen die bisherigen Veränderungen in der Landwirtschaft gestoppt werden. Dazu sind erweiterte finanzielle Anreize für biodiversitätsfördernde Landnutzung notwendig. Derartige finanzielle Anreize sollten nicht nur eine hohe Biodiversität innerhalb von Parzellen fördern, sondern auch zwischen den Parzellen. Deshalb sollten diese Zahlungen nicht nur auf die Nutzung einzelner Parzellen abzielen, sondern auch die Vielfalt an Landnutzungstypen verschiedener Parzellen eines Betriebs oder einer Gemeinde berücksichtigen.

Die Pflanzenartenvielfalt war am höchsten in ungedüngten gemähten Flächen. Da die Mahd besonders aufwändig an steilen Hängen hoher Lagen ist, sind finanzielle Anreize besonders zum Schutz der Restbestände blumenreicher Wiesen in hohen Lagen – sogenannter Wildheuplanken – besonders wichtig. Solche Hilfsgelder sind auch gerechtfertigt, wenn die Flächen schon mehrere Jahre brach lagen und wieder mit der Landnutzung begonnen wird, wie es beispielsweise im Kanton Graubünden schon erfolgreich durchgeführt wird.
Im Gegensatz zu den oft verbreiteten Vorbehalten gegen Beweidung konnten wir zeigen, dass besonders extensive Beweidung verschiedene positive Seiten hat, sowohl auf Landschafts- und Artenebene, als auch im Hinblick auf innerartliche Diversität und die Förderung biologischer Interaktionen. Aus Sicht der Biodiversität ist Beweidung von Flächen eindeutig der Verbrachung vorzuziehen. Insgesamt, zusammen mit gemähten Flächen, tragen beweidete Flächen bedeutend zur Biodiversität von Grasland in den Alpen bei.

Zusammenfassend können wir feststellen, dass zur Förderung der Biodiversität auf allen Ebenen eine möglichst vielfältige Landschaft erstrebenswert ist. Um dies zu erreichen sollten finanzielle Anreize auf eine Vielzahl an Landnutzungstypen abzielen, die möglicherweise am besten auf Gemeindeebene erreicht werden kann. Die zunehmende Monotonisierung in der Landwirtschaft oder sogar die Abwanderung aus Regionen wird drastisch die Biodiversität reduzieren. Gleichzeitig vermindert sie die Anziehungskraft für

Touristen und gefährdet zudem die Überreste des Kulturerbes in den Schweizer Alpen. Die Erhaltung der Biodiversität auf allen Ebenen biologischer Integration und ihrer kulturellen, ästhetischen, ökologischen und ökonomischen Werte erfordert eine Aufrechterhaltung der vielfältigen Landschaft der Alpen.

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